*The prevalence **of extramucosal** fungal elements in eosinophilic chronic rhino sinusitis (CMS) with bilateral paly posh confirmed by **updated mycologic** and histologic **diagnostic** methods*

*A thesis submitted for partial fulfillment **of MD.** degree **in** otorhinolaryngology*

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Page

List of Tables

List of Figures

List of Figures

Glossary of **Mycologic** Terms.

Anamorph: Asexual or imperfect state of a fungus. Identification is based on mitotic or asexual reproductive structures.

Conidia: Asexual reproductive structures (spores) produced either from the fransformation of a vegetative yeast or hyphal cell or from a specialized conidiogenous cell, which may be simple to complex and elaborate. Conidia may be formed on specialized hyphae, termed *conidiophores. Microconidia* are small, and *macroconidia* are large or multicellular.

Annelloconidia: Conidia that are produced in succession by a conidiogenous cell, called an *annellide,* which acquires an apical annulation with the formation of each conidium (e.g., *Exopliiala)*

Arthroconidia (arthrospores): Conidia that result from the fragmentation of hyphal cells (e.g., *Coccidioidcs inun ills).*

Blastoconidia (blastospores): Conidial formation through a budding process (e.g., many yeasts; Cladosporiumn species).

Chlamydospores (chlamydoconidia): Large, thick-walled, usually spherical conidia produced from terminal or intercalary hyphal cells (e.g., Candida albicans).

Phialoconidia: Conidia that are produced by a vase-shaped conidiogenous cell termed a phialide (e.g., Aspergillus fumigatus, Phialophora verrucosa).

Dematiaceous: Fungi whose cell walls contain melanin and possess a brown to black pigment.

Dimorphic: Fungi that have two growth forms, such as a mould and yeast, which develop under different growth conditions (e.g., Blastomyces dermatitidis forms hyphae in vitro and yeasts in tissue),

Hyphae: Tubular, branching filaments (2 to 10 pm in width) of fungal cells, the mould form of growth. Most hyphal cells are separated by porous cross-walls, or septa, but the zygomycetous hyphae are characteristically sparsely septate. Vegetative or substrate hyphae anchor the colony and absorb nutrients. Aerial hyphae project above the colony and bear the reproductive structures.

Imperfect fungi: Fungi that lack sexual reproduction; they are represented only by an anamorph.

Mould : Hyphal or mycelial colony or form of growth.

Mycelium ; Mass or mat of hyphae, mould colony.

Perfect fungi: Fungi that are capable of sexual reproduction.

Pseudohyphae: Elongated chains of buds or blastoconidia.

Septum: Hyphal cross-wall, typically perforated.

Sexual reproduction and spores: During sexual reproduction, haploid cells of compatible strains of a species mate through a process of plasmogamy, karyogamy, and meiosis.

Ascospores: Following meiosis, an ascomycete normally produces four to eight ascospores form within an ascus. Asexual reproduction in the ascomycetes is

through conidia. Moulds have septate hyphae. Examples: Ajellomyces (anamorphic genera, Blastomyces, Histoplasma), Arthroderma (anamorphic genera, Microspora in, Trichophyton), and yeast genera, Saccharomyces, Hansen ula.

Basidiospores: Following meiosis, most basidiomycetes produce four spores that usually form on the surface of a specialized club-shaped structure, the basidium.

Zygospores: Following meiosis, zygomycetes produce a large, thick-walled zygas pore. Asexual reproduction occurs through sporangia. Vegetative hyphae are sparsely septate. Examples: Rhizopus, Absidia, Mucor, Cunninghamella.

Sporangiospores: Asexual structures characteristic of zygomycetes; they are mitotic spores (mitospores) produced within an enclosed sporangium, supported by a specialized aerial hypha or sporangiophore.

Spore: A specialized structure with enhanced survival value, such as resistance to adverse conditions or features that promote dispersion. Spores may result from asexual (e.g., conidium and sporangiospore) or sexual reproduction.

Svnnema: Compact grouping of elongated conidiophores, producing conidia at their tips.

Teleomorph: Sexual or perfect state of a fungus.

Yeasts: Unicellular, spherical to ellipsoidal (3 to 15 pm) fungal cells, most of which reproduce by budding.

AIM OF WORK

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The aim of this work is to define the true incidence of fungal elements in the nasal and sinus mucous in cases of chronic rhinosinusitis (CRS) with bilateral polyposis compared with normal controls via mycologic and histologic identification of the various fungal species colonizing the viscid allergic mucin using recent handling techniques in collecting this mucin harboring these fungi.

REVIEW OF LITERATURE

Review of literature

Chronic rhinosinusitis can be defined as inflammatory disease of the nasal and pararasal mucosa persisting and symptomatic for more than 3 months with objective findings of mucosal inflammations including: Granulation tissue, polyp formation and fibrosis. In clinical practice it is also important to realize that mucosal changes usually persist for at least 8 weeks after treatment of an acute infection and it is not clear to which extent this mucosal damage is reversible **(Melen** et,ai» 1986).

Recently interest has been focused on local mucosal inflammation encountered in chronic rhinosinusitis, especially on the inflammatory cells whose immunoactive products seem to keep inflammation ongoing even after the infection is cured (Melen, 1994).

Chronic rhinosinusitis causes 90% of all cases of rhinosinusitis. The disease (CRS) is dramatically on the rise and has become the most common disease in the United States, with **an** increase in prevalence of 60.3% over a 12-year period (1982-1994) and 37 million patients (14.13%) of population afflicted **(Braun et al, 2003).**

In Europe, there are no data available but rhinitis and chronic rhinosinusitis are two of the most common reasons for individuals seeking medical care. **(Scheuiler et.al., 2004).**

Nasal polyposis has been an enigma in the recorded history of man kind. The history of nasal polyps goes back for a period of over 4,000 years. This condition may perhaps be the earliest disease on record for which we know the names of both the patient and physician. **(Brain 1997).**

The condition was first recognized in ancient EGYPT with the ancient Egyptian skulls showing the grosser features of nasal

polyps. Hippocrates (BC 460-370) recognized them as well and devised a method for their treatment; it is worth saying that the word polyp comes from the Greek, although it was subsequently Latinized and means many-footed (polyponus). (**Vancil** 1969),

All polypoidal conditions were initially grouped together until the middle of the nineteenth century when Billroth was able to describe the histological classification of nasal polyposis that helped to differentiate them from the neoplastic conditions. (Berda! 1954).

In 1882, Zuckerkandl, understood that nasal polyps were an inflammatory condition and he demonstrated that the histological changes in the sinuses were the same as those in the polyps, pointing out that the nasal polyps most commonly originated from the lateral nasal wall in the ethmoidal clefts. **(Zuckerkandl 1882).**

The etiology of nasal polyposis was a subject of great conflict between the authors; some of the most commonly reported causes of nasal polyps include infection, allergy, immunologic factors, metabolic diseases, hereditary diseases and autonomic dysfunction. **(Drake-Lee 1994).**

A new etiologic factor was introduced by Safirstein in 1976 who noticed the combination of nasal polyposis, thick tenacious mucin, crust formation and sinus cultures yielding "Aspergillus" and this started the era of fungal condemnation and gave rise to several descriptive nomenclatures such as "Allergic aspergillosis of the para nasal sinus"(Mi!lar **1981).** "Allergic aspergillus sinusitis" **(Katzenstein et al., 1983).** "Allergic fungal rhinosinusitis" **(Robson et al., 1989).**

In 1999, **Ponikau** et al presented their Mayo clinic experience that showed that most patients with Chronic Rhino Sinusitis (CRSj actually meet their verified criteria for allergic fungal sinusitis and they stated a new theory for the pathogenesis, that gave rise to the term "Eosinophilic Fungal Rhinosinusitis"(EFS) **(Ponikau** et al., 1999). .

This new theory was presented during the 1999 fall meeting of the American Rhinologic Society (New Orleans) and it was discussed widely during the XVII th World Congress of the International Federation of Oto-Rhino-Laryngological Societies (IFOS) held in Cairo 2002, although this theory did not gain the acceptance of all the polyposis minded rhinologists however it provided a new hope for those who are suffering from this dilemma.

I-Epidemiology, origin, aetiology, pathogenesis,, and pathology of of chronic rhinosinusitis with polyposis.

1- Epidemiology:

A- Incidence:

The true incidence is difficult to asses but it may be calculated from the incidence of nasal polyps in late onset asthmatics. The incidence of asthma is about 5% of the whole population. Where 0.1-0.5% has nasal polyps then it would be expected that between 1 in 1000 and 20 in 1000 of the adult population would have nasal polyps once or more in their life. **(Drake-Lee 1984).**

B-Sex:

There is a strong male predominance in patients who have chronic rhinosinusitis with polyps; figure range between 2:1 to 4:1 depending on the study. The male predominance is present also in children with cystic fibrosis. The sex incidence of patients with asthma and nasal polyposis is equal which suggests a different clinical subgroup. **(Drake-Lee 1997).**

C-Age:

Simple polyps may arise at any age after the age of 2 years, and if seen before this age, a meningocoele or encephalocoele should be excluded. It is unusual for simple nasal polyps to arise before the age of 10 years and if found may be the presenting complaint of cystic fibrosis. **(Schwachman 1962).**

Benign simple polyps generally present in adults and are rare before age 20 years, between 20 and 60 years of age, the incidence Is similar in each decade of life. It is generally believed that the incidence of polyps decreases after age 60 years. **(Larsen** and **Tos** 1995).

D»Genetic predisposition:

Maloney and Oliver, studied the HL-A classification of 29 patients who have chronic rhinosinusitis with nasal polyps and found that there was a higher incidence of A1/B8 in patients with nasal polyps, asthma and aspirin sensitivity. (Maloney J. and Oliver 1980).

Cystic fibrosis gene probes have been used in seven patients but without cystic fibrosis and the G551d mutation was found to be higher than expected in this small study so this work suggests that nasal polyps are associated with higher incidence of this gene mutation. **(Burger** et **aL, 1991).**

Several members of a family may be affected with nasal polyps but there is little evidence for a genetic basis for this. Some evidence to support genetic predisposition comes from the development of polyps in identical twins. **(Drake-Lee 1992).**

E-Racial groups:

Nasal polyps have been reported in all major racial groups though the comparative incidence has not been documented.
(Drake-Lee 1997).

2- Origin of nasal polyposis:

Although endoscopic and microscopic surgical techniques have been popular treatment methods for nasal polyps in recent years, there has been less attention given to the origin of nasal polyps. (Seiner, 1988). However, as far back as 1882, Zuckerkandl pointed out that nasal polyp most commonly originated from the lateral nasal wall in the region of the ethmoidal clefts. (Zuckerkandl 1882).

Stammberger in 1991 evaluated nasal polyps in 200 consecutive patients undergoing functional endoscopic sinus surgery. He noted that 80% of polyps originated from the middle meatal mucosa, uncinate process, and infundibulum. In 65%, polyps originated from the ethmoidal bulla and hiatus semilunaris and from the frontal recess in 48%. Polyps were found inside the ethmoidal bulla in 30%.

Through the period from 1991 to 1996 Larsen and Tos, evaluated the origin of nasal polyps in three cadaveric studies. In there first study, conventional anterior rhinoscopy was performed during 300 autopsies and nasal polyps were noted in six nasoethmoidal specimens with a frequency of about 2%.
(Larsen and Tos 1991)(Table 1).

In their second study in 1994, the nasoethmoidal complex was removed transcranially in 19 autopsy specimens and examined directly; anterior rhinoscopy was not performed. Polyps were found in 5 of 19 specimens, a frequency of 26% **(Table** 1),
(Fig. 1). (Larsen et al.,1994)

In their third study, 31 autopsy specimens were evaluated with endoscopic dissection. Polyps were noted in 13 of 31 (42%) specimens. **(Larsen and Tos 1996) (Table 1).**

Important characteristics of nasal polyps were noted in the three studies. Polyps were found mainly in the transition spaces between the nose and sinuses. They found that 75% of polyps were related to ethmoidal recesses and clefts Most of the polyps were unilateral (63%), and bilateral nasal polyps were found in *31%* of the cadavers. (Table 2), (Fig. 2)

Screening method	*Total autopsies*	*Autopsies with polyps*	*Frequency (%)*
Rhinoscopy speculum	300	6	*2*
Transcranial bloc removal	19	5	26
Endoscopic surgery	31	13	42

Table-1 Frequency of nasal polyps in three screening methods on consecutive autopsies. Quoted from **Tos** and **Larsen.2001.**



Fig.l Nasoethmoidal block. A small polyp (P) originates from the ethmoidal cleft in the superior meatus. A bougie is placed in the posterior ethmoidal cell recess. P = polyp; S = septum; M = middle turbinate;arrow = superior turbinate; A = anterior. Quoted from Larson and Tos 1995.

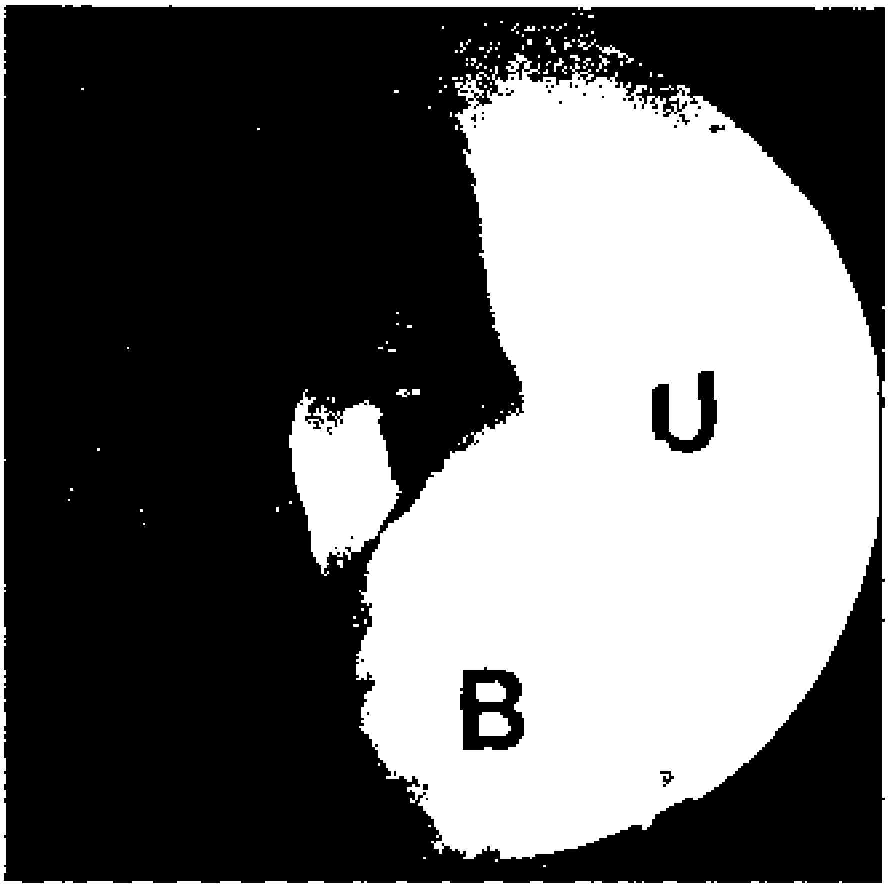


Figure 2 Small stalky polyp orgmating in the anterior part of the left frontal recess. Endoscopic view. Arrow = polyp; M = middle turbinate; B = ethmoidal bulla; U = uncinate process. Quoted from Larsen and Tos 1996.

Origination	*Relation to Sinus Ostium*	
	No	Yes
MMm (middle turbinate)	4	-
MM1 (uncinate process)	1	-
MM1 (bulla)	1	-
MM1/1 (frontal recess)	-	4
MM1 (anterior ethmoidal cleft)	-	12
MM1 (maxillary sinus ostium)	-	4
SMI/1 (posterior ethmoidal cleft)	-	7
Middle turbinate	1	–
Agger nasi	1	
Sphenoethmoidal recess	-	1
Total	8	28

MM = middle meatus; SM = superior meatus; m = medially; 1 = laterally; t = top.

Table 2 Origination of nasal polyps in 18 autopsy cases, 5 transcranial blocks, 13 endonasal surgery. Quoted from **Tos and Larsen.2001.**

3- Aetiology of nasal polyposis:

The etiology of nasal polyps is multifactorial. It is widely accepted that there may be more than one cause. Masai polyps are not a disease but a physical finding with a number of causes. Some of the more commonly reported causes-of nasal polyps include infection, allergy, immunologic factors, metabolic diseases, hereditary diseases, and autonomic dysfunction. Ciliary abnormalities, as found in primary ciliary dyskinesia can be associated with polyps as can the hyperviscous mucus in Young's syndrome. (Drake-Lee 1994).

Patients with nasal polyps may be grouped clinically into two groups: patients with panrespiratory disease and patients with nasal disease alone. Patients with panrespiratory disease, such as iate-onset asthma, cystic fibrosis, immune deficiency, and primary dyskinesia, are the most difficult to treat either medically or surgically. **(Larsen et aL, 1992).**

Patients with nasal disease alone are easier to treat but are more difficult to evaluate, since several factors in the nose may contribute to the presence of nasal polyps. Factors include chronic infection of the sinuses, extensive mucosal disease, and anatomic abnormalities, especially in the middle meatus.(Tos **and Larsen 2001).**

Hereby, some of the more commonly cited **causes of nasal polyps,** including **chronic infection, allergy, eosinophilic fungal rhinosinusitis.**
A-Chronic infection:

In patients with nasal polyps, chronic infection of the nose and paranasal sinuses frequently is present. The most common pathogens are *f-hemolytic streptococci, Staphylococcus aureus. Streptococcus pneumoniae,* and *Haemophilus influenzae.*
(Majumdar and Bull 1982)(Table 3)

Dawes et al., in 1989 found that pus cells and bacteria were only

found in 16 out of 100 antral irrigations in patients having chronic rhinosinusitis with polyposis. (Dawes et al.,1989).

Recent investigations of experimental maxillary sinusitis in rabbits demonstrated both granulation-like polyps and edematous-like polyps. In these studies, the sinuses were inoculated with *S. pneumoniae* type 3, *Bacteroides fragillis*, or *S. aureus*, after simple blocking of the sinus ostium. Granulation-like polyps were noted in regions of deep inflammatory trauma and edematous polyps were found in regions of superficial inflammatory trauma. Polyps were found in all sinusitis groups irrespective of an inducing agent and were not directly related to the presence of a certain microorganism. **(Norlander et al., 1993).**

Viruses also have been mentioned as a possible cause of nasal polyps. **(Weille and Gohd 1957).**

However, a viral etiology has not been shown, despite investigation for adenovirus, Epstein-Barr virus, herpes simplex virus, and human papilloma virus using polymerase chain reaction and DNA hybridization. **(Kozak 1991).**

	Normal nose (Gwaltney and Hyden, 1982)		Chronic rhinosinusitis with polyposis (Dawes et ah, 1989)
	Anterior	*Posterior*	*Chronic rhinosinusitis with polyposis*
Staphylococcus aureus	30	10	-
Steptococci(all)	m	15	10
Gram-negative bacteria	*1*	10	-
Haemophilus influenza	m	40	10
Anaerobes	•	-	-

Table 3 Comparison of the % of bacterial cultures from the normal nose and chronic rhinosinusitis with polyposis, quoted from: Gwaltney and Hayden(1982), Dawes et ai.(1989).

B-Allergy:

Allergy has been implicated because of three factors: the histological picture was that 90% or more of the nasal polyps have an eosinophilia, the association with asthma and finally, the nasal findings which may mimic allergic symptoms and
signs.(Drake-Lee 1997).

In patients with a history of allergy and nasal polyps noted on examination, the inflammatory infiltrate of the nasal mucosa often contains eosinophils. The eosinophil leukocyte is the dominant cell in polyps and smears of nasal mucus. Therefore, allergy has been considered an important etiology for nasal polyps. Kern and Schenck, reported nasal polyps in 25.6% of

patients with allergy compared to 3.9% of a control group without allergy. (**Kern** and **Schenck** 1993).

The prevalence of allergy in patients with nasal polyps varies from 10% to 54% and 64 %.(**Blumstein** and **Tuft** 1957)

There is a coincidence of asthma and nasal polyps, but the association between polyps and atopic allergy is not clear. Patients with nasal polyps have the same prevalence of positive skin prick tests to a series of allergens as the normal population.(Pepys and **Duveen** 1951).

The mechanisms involved in eosinophilic infiltration of the nasal mucosa and the nasal polyp are not fully elucidated. Studies on tissue cultures of epithelial cells from nasal polyps and normal nasal mucosa, generating human epithelial cell- conditioned media, suggested that eosinophil infiltration into the nasal mucosa during allergic reaction and nasal polyposis may be modulated, at least in part, by epithelial cells. Epithelial cells from nasal polyps might have a more potent effect on inducing eosinophilic infiltration of the respiratory mucosa than epithelial cells from healthy nasal mucosa. **(Xaubet et** aL, **1994).**

Other studies showed that eosinophil migration into tissues is controlled, in part, by interactions between eosinophil adhesion receptors and counterstructures on the vascular epithelium. This p-selectin-dependent adhesion is important.
(Symon, 1994).

On the other hand, Mygind believes that most clinical evidence is against allergy as a causal factor. **(Mygind 1978).**

C- Allergic **fungal rhinosinusitis:**

In 1983, Katzenstein and colleagues described allergic *Aspergillus* sinusitis as a newly recognized form of sinusitis. The diagnosis was made based on the histologic triad of (I) clumps' or sheets of necrotic eosinophils; (2) Charcot-Leyden crystals (from degraded eosinophils); and (3) noninvasive fungal hyphae with morphology consistent with *Aspergillus* species within the nasal

mucus,(Katzenstein et **al.,1983).** In 1989, Robson and colleagues introduced the term *allergic fungal sinusitis* (AFS) because they identified a number of fungi thought to cause the same disorder. (Robson et al., 1989). In 1990, Ence and colleagues identified 5 different organisms responsible for AFS. (Ence et **al.,1990).** Cody and colleagues reported that *Aspergillus* species were responsible for only about 15% of cases of AFS in a large retrospective study. **(Cody et** al., 1994).

The incidence of AFS in cases of chronic rhinosinusitis (CRS) treated surgically has been approximately 6% to 7%.Nasal polyps were found in 75% and asthma was found in 65% of the AFS cases described.(Morpeth,1996)

Most authors agree that AFS is an underdiagnosed entity and that only an increased awareness among physicians to look A fungal involvement will increase the accuracy of diagnosing AFS. Unfortunately, previous diagnostic methods seem to lack sensitivity. For example, in the past, even when fungal hyphae were clearly identified in histologic specimens, only 60% of the cultures were positive for fungi. **(Cody et al., 1994).**

Based on the clinical findings in 16 patients, Bent and Kuhn proposed 5 criteria for the diagnosis of AFS; (1) nasal polyposis; (2) allergic mucin; (3) computed tomographic (CT) scan findings consistent with CRS; (4) positive fungal histology or culture; and (5) type I hypersensitivity (atopy) diagnosed by history, positive skin test, or serology.**(Bent and Kuhn 1994).**

Recently, De-Shazo and Swain described 7 patients with AFS in whom they applied similar diagnostic criteria, with the exception of atopy. The reason they excluded atopy as a diagnostic criterion for AFS was their review of the literature in which they found that only two thirds of patients tested had a positive skin test result to the fungi cultured. In addition, 1 of their 7 patients with the histologic diagnosis of AFS had no evidence of atopy.(De-Shazo and Swain 1995).

Thus, the diagnostic criteria for AFS are (1) CRS; (2) the

presence of allergic mucin (clusters of eosinophils and their by-products, eg, Charcot-Leyden crystals and major basic protein); and (3) the presence of fungal organisms within that mucin, confirmed by histology, culture, or both.(Ramadan and **Quraishi 1997).**

The exact pathophysiology of AFS remains a matter of depot for which several theories have been offered. One popular theory proposed by Manning and Holman is based on the assumption that AFS exists as the nasal correlate of allergic bronchopulmonary Aspergillosis, and suggests that several interrelated factors and events lead to the development and perpetuation of the disease. First an atopic host is exposed to fungi through normal nasal respiration, thus providing an initial antigenic stimulus. Gel and Coombs type I (IgE) and III (Immune complex) mediated reaction then trigger an intense eosinophilic inflammatory response. The resulting inflammation leads to obstruction of the sinus ostia, resulting in stasis within the sinuses. This in turn provide an ideal environment for further profilation of the fungus, thus increasing the antigenic exposure. At some point this cycle become self-perpetuating and results in the eventual products of this process: allergic mucin, the material that fills the involved sinus of patients with AFS. Accumulation of the allergic mucin obstructs the involved sinuses and propagates the process. **(Manning and Holman 1998).** (Fig. 3).

Ponikau and colleagues in 1999, demonstrated the extensive presence of fungi within the nose and paranasl sinus through the use of an extremely sensitive fungal culture technique. Their report from the Mayo Clinic revealed that the histologic markers of AFS are the striking numbers of eosinophils in mucus of the patients, in contrast to the near absence of eosinophils in healthy controls. They demonstrated that eosinophils, when present in high numbers in the tissue in CRS, are also invariably present in the mucus, mostly in the form of cell clusters. They concluded from this observation that the eosinophils are only in transit through the tissue toward the mucus and that eosinophils actually migrate intact through the epithelium and degranulate within the mucus, hypothesizing that the eosinophils play an immunologic defensive role in CRS, and their target is located in the mucus. In other words, the fungal organisms in the mucus could be the target for the eosinophils. They concluded that IgE- mediated inflammation is not crucial to the development of AFS, and that eosinophilic chemotaxis and activation may result from a T lymphocyte mediated inflammatory cascade. (Ponikau **et al., 1999).**

Although it appears clear that eosinophils play an important role in the development of both AFS, the factors that ultimately trigger eosinophilic inflammation remain in question. Indeed, eosinopilic inflammation may occur as a final common pathway in response to a number of different inflammatory starting points. It is likely that initiation of the inflammatory cascade leading to AFS is multifactorial event, requiring such things as IgE-mediated sensitivity (atopy), specific T-cell HLA receptor expression, exposure to specific fungi, and aberration of local mucosal defence mechanism **(Marple 2001). (Fig.3)**

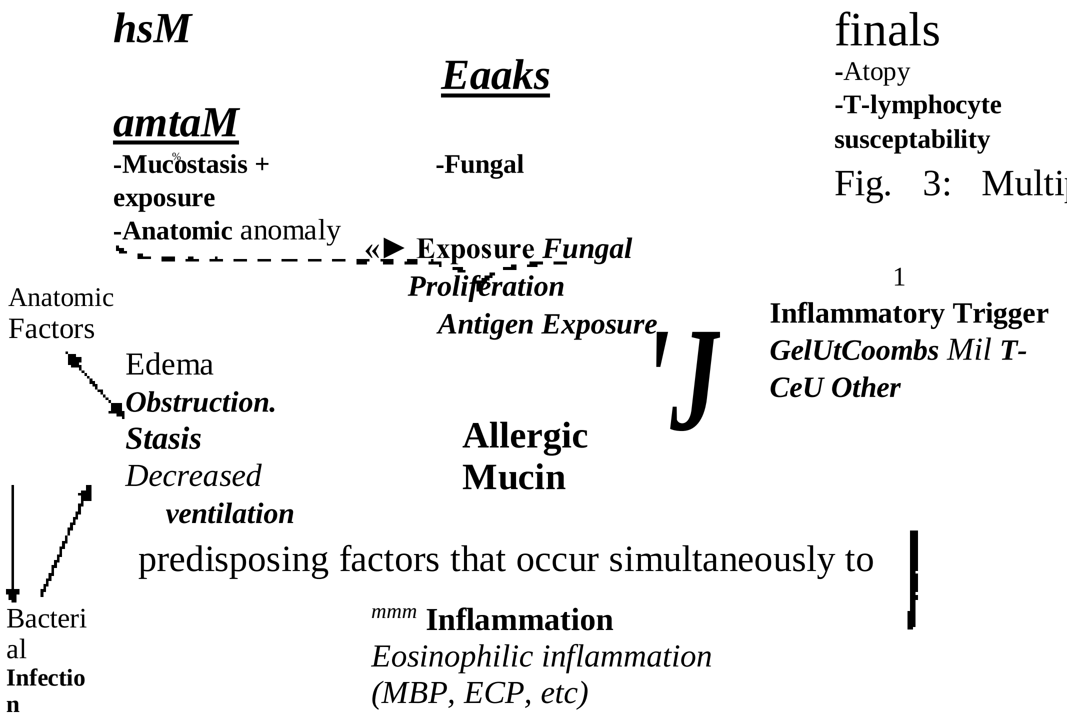


Fig. 3: Multiple predisposing factors that occur simultaneously to initiate the cyclic inflammatory cascade responsible for AFS. Quoted from Marple 2001.

4-Pathogenesis of nasal polyposis:

The major differences between nasal polyps and normal nasal mucosa are: (1) Eosinophilia, (2) Oedema, (3) Alteration in epithelial regrowth, and (4) Development of new gland formation. (Bernstein 2001).

It is emphasized that the histopathology of nasal polyps is different from that of nasal mucosa. Therefore, any theory describing the development of nasal polyps must consider the alterations in the above-mentioned characteristics. The critical point to be made is that nasal polyposis is not mere edema of normal nasal mucosa but a new inflammatory development that is the result of inflammatory mediators, cytokines, adhesion molecules, and endothelial counter-receptors.(**Bernstein 2001).**

Many theories afforded for the pathogenesis of nasal polyposis have been mentioned in the literature, the most popular theories are :

A- Type I and III hypersensitivity reaction:

This theory describes an altered host reactivity to an inhaled antigen, the immune reactions involved causes tissue damage and are mainly mediated by the immunoglobulin IgE which is attached to the mast cells. Two molecules of the allergen specific IgE need to be adjacent and the reaction with the allergen causes the cell membrane to alter and calcium ions to enter the mast cell resulting in degranulation with subsequent release of prostaglandins and leukotriens that will attracts the eosinophils and lymphocytes to the site of the reaction. It is postulated also that mast cells may be triggered by other reactions including those involving IgG and complement activation. **(Drake-lee 1993).**

Although, this theory was the first explanation of the tissue eosinophilia in the nasal polyps yet since skin tests become reliable, many authors have reported that, in fact positive skin tests are as frequently observed in nasal polyp population as in the normal population, questioning the . role of the allergy.(Frenkiel **et** aL, 1985).

Many authors have tried to find arguments supporting the hypothesis that allergic mechanisms, i.e specific IgE production and mast cell degranulation occur only locally in the nose.(Ganzer and **Bachert** 1988). Meta-analysis study, based on 9 studies (287 patients) and aimed at the detection of specific IgE in polyp secretions and mucosa, showed that only 19% of the patients without systemic allergy demonstrated local specific IgE. **(Shatkin** et al. **,1994).Summing** up the above mentioned data indicates that IgE-mediated allergy seems to play only a minor role in the patho-physiology of nasal polyposis, and they certainly do not explain the accumulation of eosinophils. **(Jankowski 1996).**

B- The autocrine hypothesis of eosinophils accumulation:

In 1979, Mullarkey and colleagues introduced the concept of noil-allergic rhinitis with eosinophilia (NARES), giving the eosinophils a central role in inflammatory process associated with chronic rhinosinusitis. Patients with NARES have a negative history of allergen-induced symptoms and negative skin and RAST tests but are characterized by having a significant number of eosinophils in their nasal secretion. (Mullarkey et **al.,** 1979).

In this concept the question remained, however, why eosinophils accumulate into the nasal polyp tissue. An interesting answer based on mediator studies has been proposed by researchers of the MacMaster University, Ontario, Canada termed **"Microenviromental theory of inflammation".** This theory proposed that tissue microenvironments are inductive compartments in which immune-effectors and inflammatory cell functions are modulated. The starting point was that those authors could demonstrate the presence of inflammatory progenitors cells in human nasal mucosa.**(Otsuka et al.,1987).**

Denburg and colleagues studied a number of cytokines that are present in nasal polyps, including interleukin (IL)-lp, tumor necrosis factor-alpha (TNF-a), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular cell adhesion molecule-1 (VCAM-1), a4pl integrins (VLA-4), lymphocyte function associated antigen-1 (LFA-1), (aLp2) and intercellular adhesion molecule-1 (ICAM-1). All these cytokines have a direct or indirect effect on the rolling, adherence, and transmigration of eosinophils from the microvasculature into the lamina propria of nasal polyps.(Denburg **et al.,1990).(Fig. 4).**

Once the eosinophils are present in the interstitium of the nasal polyp, they become activated, degranulated, and, in addition to releasing inflammatory mediators, which will be mentioned below, also release the cytokines IL-3 and IL-5. These two cytokines are responsible for increased recruitment of eosinophils in an autocrine upregulated fashion. Furthermore, these cytokines are responsible for decreasing apoptosis, leading to increased survival of eosinophils. **(Morita** et ai, 1996),

VCAM-1 is increased on the lining endothelium of mediumsized blood vessels of nasal polyps, compared with patients' inferior turbinates (Fig, 5). There is also an increase in the avidity of VLA-4 to VCAM-1 in nasal polyps and this appears to be controlled by GM-CSF. **(Sung et al., 1997).**

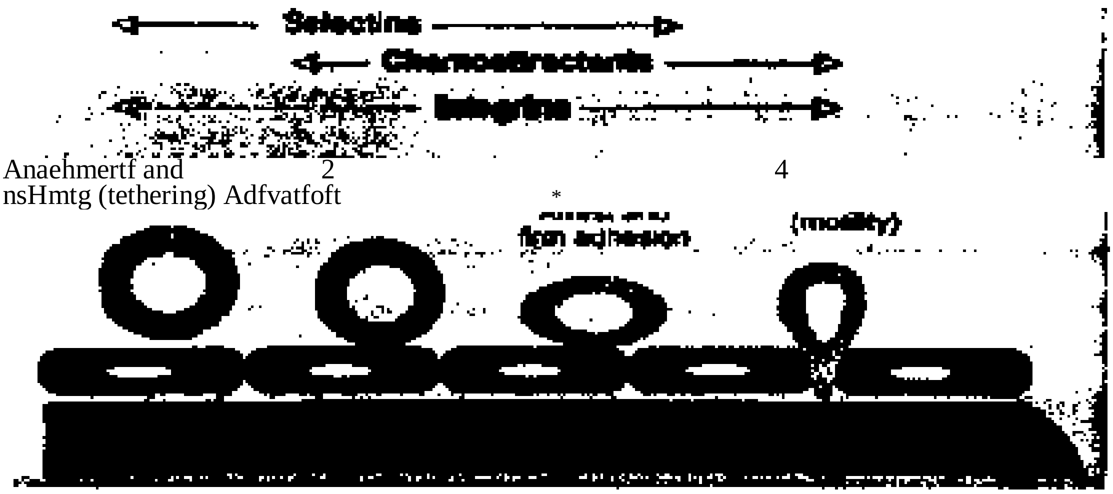


Fig. 4. Diagram of the overlapping role of selectins, chemoattractants, and integrins in leukocyte attachment, rolling, firm adhesion, and extravasation. Quoted from **Tos and Larsen .2001.**

The mechanisms of inflammatory eosinophil recruitment have been well studied and appear to involve a multistep model for leukocyte extravasation, as summarized in **(Fig.-4).** In the first step, free-flowing leukocytes within the lumen of venules attach to endothelial cells. This initial adhesion is mediated by the selectin family of adhesion molecules that recognize specific cell-surface carbohydrates. The three members of the selectin family are L-selectin expressed on circulating leukocytes, and fraud P-selectin expressed on activated endothelium. This initial adhesion is independent of leukocyte activation and is relatively loose under sheer stress; it enables slow rolling of interacting leukocytes along the vessel wall. In the second step, activation of integrins on rolling leukocytes is initiated by G protein- coupled chemoattractant receptors.**(Murphy** 1994)

Fig. 5 : Vascular cell adhesion molecule-1 (VCAM-1) is shown on the lining endothelium of a medium-sized blood vessel in the nasal polyp(closed arrows). From Tos M. and Larsen P.L.2001.

In this process, chemokines are believed to complex with and be presented by endothelial glycosaminoglycans, thereby triggering, within seconds, firm adhesion of the rolling phase of leukocytes through integrin activation. VLA-4 integrins are expressed on lymphocytes, monocytes, and eosinophils but not on neutrophils. Recent reports have shown that a4 integrins not only mediate firm adhesion but also are involved in the initial rolling phase of leukocytes, which suggests that even greater diversity exists in the process of extravasation.(Alon ef **al.,1995).**

When the leukocyte finally adheres firmly, subsequent pericellular migration through the endothelial junction occurs. This event probably is directed by a chemotactic gradient formed by chemoattractants in combination with leukocytebinding to the platelet/endothelial cell adhesion molecule (PCAM-l).(Liao et al.,1995).

Prior to the activation of eosinophils on the blood vessels, it is necessary for the counter-receptors on the endothelial cells to be upregulated. This upregulation appears to be the function of IL-ip and TNF-a. The messages for IL-ip and TNF-a are upregulated in the constitutive cells of the nasal polyp, including the epithelial cells, endothelial cells, and fibroblasts.(Liao et al.,1995). (Fig. 6,7).

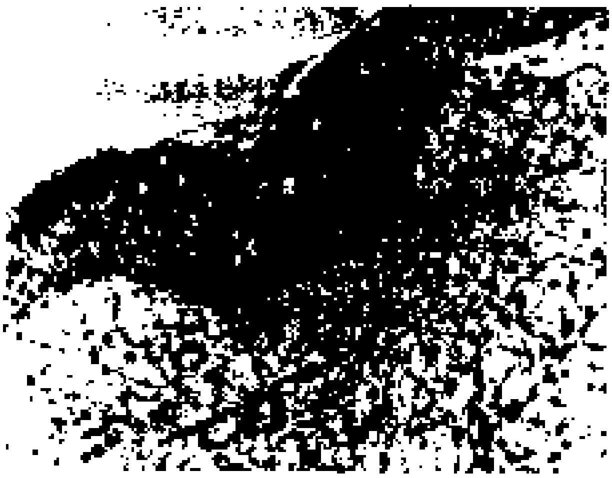


Fig. 6 : Photomicrograph of nasal polyp demonstrating TNF- a in the epithelium of the nasal polyp .Quoted from Tos. and Larsen 2001.

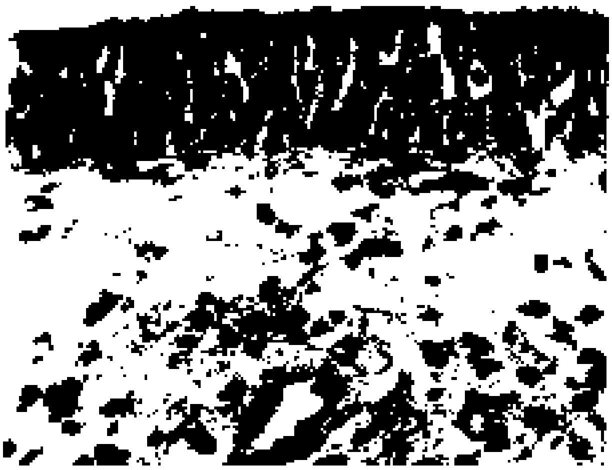


Fig.7 : IL-ip is seen in the epithelium of nasal polyp. Quoted
from Tos. and Larsen 2001.

C-Remodelling of the human nasal polyp epithelium:

Recently, it has been shown that injury to the airway epithelial cells by allergens, viruses, or trauma may lead to reconstruction of the airway epithelia in the form of basal cell hyperplasia, squamous metaplasia, and goblet cell hyperplasia. These three alterations in the regeneration of injured epithelia of nasal polyps can result in defective cystic fibrosis transmembrane regulator protein (CFTR) location and are phenotypically similar to the genetic defect in cystic fibrosis (CF). Thus, in nasal polyposis, defective sodium absorption through the amiloride-sensitive apical sodium channel may occur, even in the absence of CF. (Dupuit et **al.,1995).** (Figs, 8, 9,10,11).

The dysfunctional CFTR protein then will have an effect on the regulation of the sodium channels by increasing the number of open channels. It also will have an effect on decreasing the outwardly rectifying chloride channel (ORCC). **(Jovov et al.,1995)**

There is evidence that the eosinophilic mediator major basic protein (MBP) has an effect on altering the amount of mucus secreted by the respiratory epithelium, and furthermore, MBP has an effect on increasing chloride secretion and increasing sodium absorption.QLundgren **et al.,1991).**

All these events could alter the surface epithelium of the non-CF polyp. In as much as the CFTR protein may not be functioning, chloride secretion may be decreased, and sodium chloride then would be pumped into the interstitium. Then water would follow this increased movement of sodium into the lamina propria.This movement of sodium could account for the movement of water with resultant edema, and it is edema that is a major pathologic feature of nasal polyposis.**(Dupuit et al.,1995).**



FIg.8. Classification of different morphologic subtypes of the urface epithelium of human nasal polyps. (H&E stain , original magnification x200). Quoted from Dupuit et al.,1995.

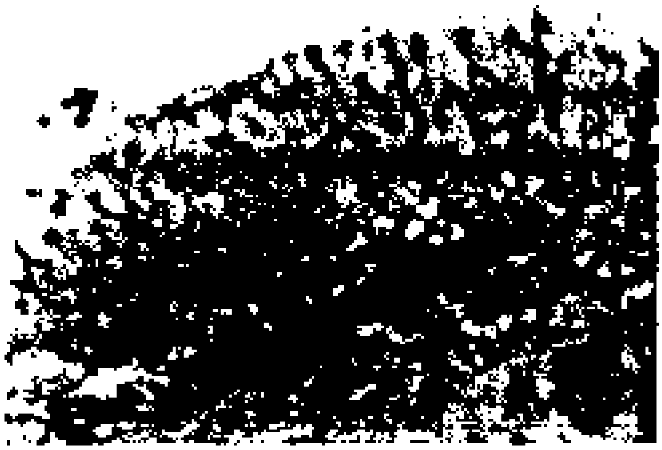


Fig.9 Basal cell hyperplasia representing at least three layers of basal cells in the epithelium of nasal polyp. (H&E stain , original magnification x200). Quoted from Dupuit et al.,1995.



Fig..lO Mucous cell hyperplasia in which the entire mucous membrane has been replaced by goblet cells. There is slight hyperplasia of the basal cell layer as well. (H&E stain , original magnification x200). Quoted from **Dupuit** et al.,1995.



Fig.ll. Squamous metaplasia characterized by the presence of multilayered flattened cells. (H&E stain , original magnification x200). Quoted from **Dupuit et al.,1995.**

5-Pathology of chronic rhinosinusitis with polyposis:

The pathophysiology of sinusitis involves many disturbed host and microbial factors of varying significance, depending on the degree of disease. Complex events such as immunologic defense, mucociliary clearance, and immune response seem to be impaired at a very early stage of sinonasal disease. Retention of secretion together with mucosal edema will reduce ostial patency, thereby initiating a sequence of events leading to disturbed cellular metabolism, inflammation, and the development of gross pathological changes. (Stierna **2001)**

I- Mucosal Lesions

Our knowledge of the early mucosal changes in sinusitis is based mainly on findings from experimental studies in rabbit animal models. Five days after induction of experimental sinusitis, either by mere ostial occlusion or with *Streptococcus, pneumoniae,* the epithelium is strongly edematous with large areas of desquamation and squamous metaplasia. Goblet cells, normally rare in the rabbit sinus mucosa, have increased in number. A progressive loss of ciliated cells, clumping of cilia, and cystic spaces in the epithelium all occur during the first 5 days of induced sinusitis. Subepithelial basement membrane thickening due to deposition of collagen and other connective tissue components develop early during infection and may impair the transport of fluid and nutrients to the epithelium while negatively influencing the differentiation of the regenerating epithelium. Obviously, these types of lesions add to the cycle of functional impairment during sinusitis. After 2 weeks of sinusitis induced with *S. pneumoniae* (purulent) or occlusion of the ostium (nonpurulent), the mucosa shows signs of recovery, in contrast to the findings in experimental sinus infection with *Bacteroides fragilis, Staphylococcus aureus,* and *Pseudomonas aeruginosa* in which pronounced epithelial changes persist for at least 12 weeks. In the latter models, mucosal changes are also more profound, frequently displaying longer periods of heavy fibrosis and further involvement of the underlying bone. Thus, it is evident that the virulence factors of an infecting organism, specific for each bacterial species, have considerable consequences for the host response. **(Bolger et al., 1997)**

II- Goblet Cells.

Most of the mucus in a normal maxillary sinus is probably produced by the goblet cells of the surface epithelium, but hyperplasia and hypersecretion of tubuloacinar glands appear during infection. It has also been postulated that glandular hyperplasia and goblet cell differentiation in the region of the anterior ethmoidal sinus and ostiomeatal complex are of importance in the mucus membrane thickening and mucus entrapment near the orifice of the maxillary sinus. The distention and regenerative response of the glandular acini with the disruption of their architecture may lead to mucoceles with mucus-generating epithelia that secrete into closed, submucosal space and become cystic. In humans, the goblet cell density of the maxillary sinus is significantly reduced, especially in purulent sinusitis, whereas experimentally induced sinusitis in rabbits leads to hyperplasis of goblet cells in the epithelium. **(Tos and Mongensen 1984)**

In experimental sinusitis, an extreme degree of inflammation may lead to a decrease in goblet cells together with gland involution accompanied by a decreasing number of glands. The goblet cell differentiation thus shows regional differences as well as being dependent on the degree of inflammation. These qualitative and quantitative changes in secretion together with the formation of cysts and atypical glands may, from different aspects, impair mucociliary transport by retarding ciliary clearance as well as by developing mucosal pathology. **(Stiema and Jannert 1994)**

Ill-Polyps

No single predisposing disease can account for the formation of nasal polyps in all patients, while an underlying inflammatory process and/or infection will lead to the development of this specific local tissue pathology. Mucosal polyps of the sinus and nasal mucosa may be experimentally created by the induction of both inflammation and infection. This involves a very specific cellular sequence bearing many similarities to the normal healing process after surface epithelial trauma. Light and electronmicroscopic studies in experimental models have shown that the initial polyp formation sequence involves multiple epithelial disruptions with proliferating granulation tissue where immature branching epithelium migrates to cover the mucosal defect. Other branches spread into the underlying connective tissue, where intraepithelial microcavities with a differentiated epithelial lining separate the

developing polyp body from the adjacent mucosa. Polyp formation and growth is thus activated and perpetuated by an integrated process of mucosal epithelium, matrix, and inflammatory cells, which in turn may be initiated by both infectious and noninfectious inflammation. Since these mechanisms of epithelial proliferation and differentiation display local variations, this may explain the regional tendency toward polyp development in the nasal cavity. Polyp formation appears to be a dynamic process where smaller sized polyps may involute, unless a persisting inflammation continues to challenge the early stage of the newly formed polyp. Once formed, a polyp’s growth is dependent on local inflammation, edema formation, and stromal cell proliferation perpetuating this localized tissue process. **(Norlander et al., 1996)**

Nasal polyps vary in size, depending on growth rate, polyp age, degree of inflammation and edema, and several other factors that include the anatomy of the meatus, the space available for its extension, and the number and size of neighboring polyps. The polyp body or apex is connected by a stalk of various thickness and length to the base of the polyp at its site of origin on the nasal mucosa (Fig, 12). A polyp is made up of several tissues. Its stroma is a reticular network that encloses an amorphous substance comprising fluid, blood vessels, glands, and inflammatory cells. The surface is covered with epithelium that lies on a poorly defined basement membrane. The inflammatory cells and the epithelium may change during the course of development and growth of the polyp. To understand the various pathogenic theories on polyp formation presented over the past several years, one needs to understand the histopathologic dynamics in polyp tissue.(Tos 1990).



Fig.12. Stalky edematous polyp removed with its connecting mucosal base. P= polyp; small arrows= nasal mucosa; one bar= 1 mm.Quoted from Tos 1990.

A-Epithelium:

The dominant epithelium type is pseudostratified columnar cylindric epithelium with goblet and ciliary cells-However, other epithelia may also be present on the polyp surface. In a systematic study of polyp epithelia and goblet cell density, one half of the polyp was stained using a PAS-alcian blue whole mount method and the goblet cell density determined on nine localities of the polyp surface. After cell density had been determined for each location, the polyp halves were serially sectioned and stained again with hematoxylin andeosin, and the epithelium type was determined at the same places **.(Krajina 1963). (Fig. 13).**

Besides the pseudostratified epithelium of various height found in the majority of cases, transitional epithelium and squamous nonkeratinized epithelium were found. Pseudostratified cylindric ciliary epithelium, characterized as hyperplastic, high, or low, was found in the majority of localities. **(Larsen and Tos 1989). (Fig. 13).**

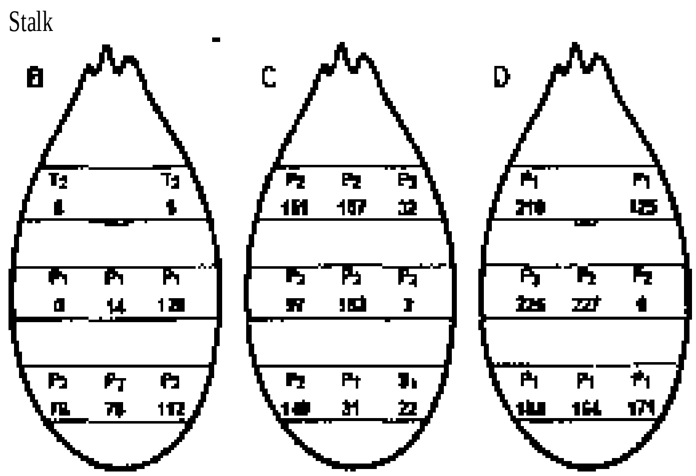


Fig. 13: Different epithelial types and height found on different levels of the nasal polyps. Density of goblet cells in the corresponding epithelial type is indicated by numbers per 0.01768 mm2 field. In four polyp halves A, B, C, and D.P = pseudostratified; T = transitional; S = squamous epithelium, 1 = high epithelium; 2 = normal epithelium height Quoted from Larsen and Tos 1989.

The density of goblet cells was highest in areas with hyperplastic pseudostratified epithelium. These areas demonstrated approximately 126 cells per 0.01768 mm^2 and corresponded to 7,000 cells per mm2. Even in low pseudostratified epithelium, the goblet cell density was high.Transitional epithelium, which was usually thick, was most [F]ten found in the anterior surfaces of the most anterior polyps **"ig. 14).**

In the anterior polyps, pseudostratified cylindrical epithelium was found in 62% of the localities, transitional ithelium in 33%, and squamous epithelium in 5%. The corresponding percentages in the posterior polyps were 78%, 19%, and 3%, respectively. This difference is statistically oignificant. **(Larsen and Tos 1989).**

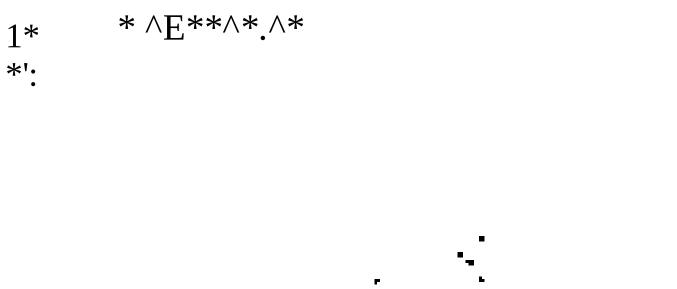
Mogensen and Tos 1977, proposed that, air current exerts a constant influence on the respiratory epithelium of the nose, possibly causing transformation to transitional or squamous epithelium in large segments of the nasal mucosa. Therefore the finding of larger areas of transitional epithelium in anterior polyps compared with posterior polyps may be caused by a greater influence of the air current on the anterior polyps. However, posterior polyps are also often exposed to air current, particularly in the apical segments, which may explain the findings of transitional epithelium and squamous epithelium in posterior polyps. Experimental closure of the nostril and thereby elimination of the air current causes a transformation of squamous and transitory epithelium into respiratory epithelium on the closed side and vice versa on the open side. Usually, transition from one type of epithelium to another is gradual, but in rare cases, the transition can be abrupt.(Larsen **et al.,1986). (Fig. 15 and 16).**



A, High pseudostratified '.y PPf ciliary epithelium with many - j* goblet cells.(original magnification *500)



B, High Pseudostratified ciliary epithelium without goblet cells (original magnification *800).



C, Low pseudostratified ciliary epithelium with few goblet cells.(original magnification x800).

Fig. 14: Examples of the relationship between epithelium type and goblet cell density. Quoted From Tos et al.,1990.

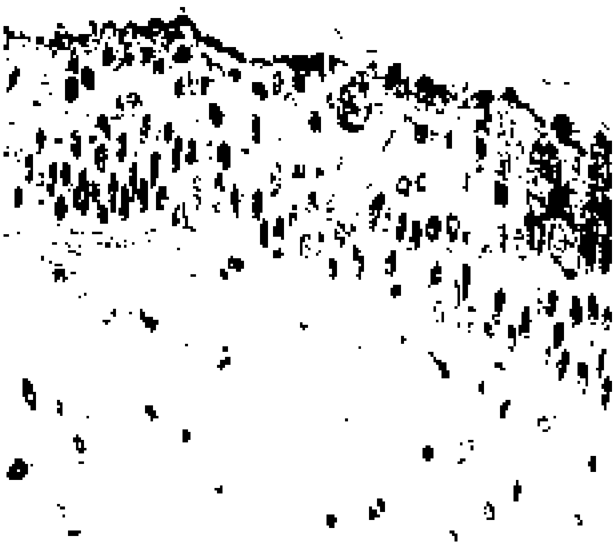


Fig.15: Gradual transition between Pseudostratified and transitional epithelium types. Quoted from Larsen et al.,1986.



Fig.16: Abrupt transition between Pseudostratified cylindric epithelium to low transitional epithelium (original magnification *350). Quoted from Larsen et al.,1986.

Analysis of goblet cell density revealed greater variation among different areas of the nose with similar epithelium. Additional analysis showed that goblet cell density also varied widely among different epithelial types. Certain areas within a polyp are completely lacking in goblet cells, whereas other show relatively high density. There are large differences in median goblet density between individual polyps. (Fig. 13 and 17). (Tos et al.,1990).

Such large differences have not been found in the mucosa of the nose and paranasal sinuses. The described variability of the epithelia and goblet cell density expresses the tremendous dynamics of histopathology in nasal polyps. Here, changes are influenced by air current that causes, significantly lower goblet cell density in the anterior than posterior polyps.(Tos and Mogensen ,1979).

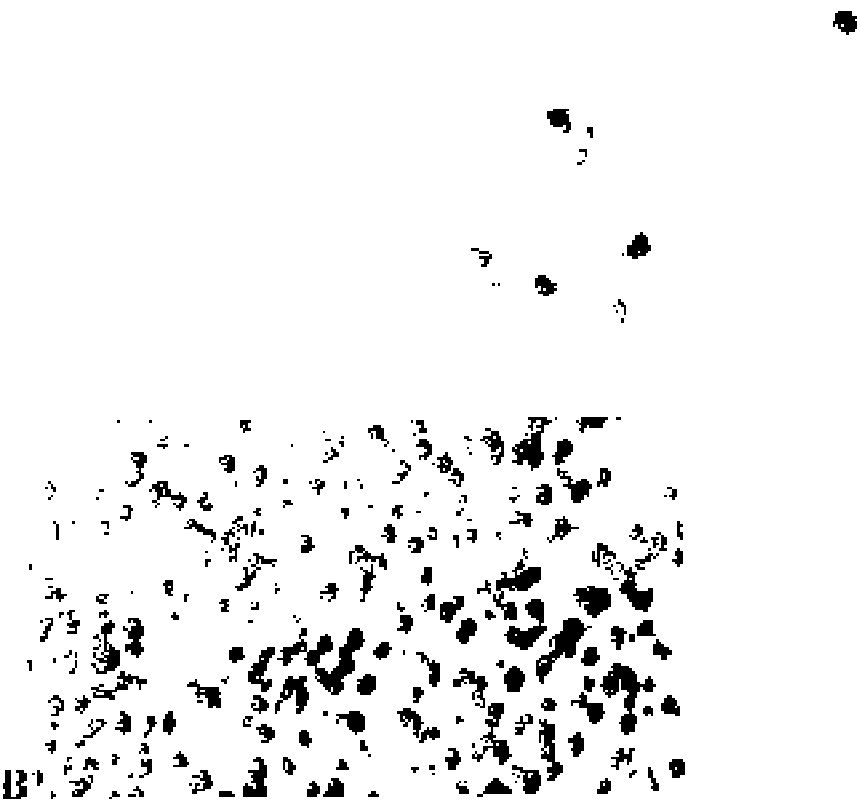


Fig.17: PAS-alcian blue whole mount specimens of nasal polyps, (original magnification ^{X}50Q). Quoted from Tos et al.,1990

A, Low density of goblet cells.

B, High density of goblet cells.

B-Stroma:

i- Mucous glands:

The presence and character of mucous glands in nasal »lyps have been the topic of discussion since the last century, "he famous surgeon Billroth, in his 1885 thesis on the "Structure of Nasal Polyps," described nasal glands as long tubular new formations. Others considered these tubular formations as nasal glands. Tos and Mogensen have thoroughly adied mucous glands in nasal polyps and demonstrated that they play an important role in the pathogenesis and growth of nasal polyps. In their study glands were found in all 102 polyps studied by whole mounts and sections, but in many, only few glands were noted. The density was considerably lower in the sinus as compared with the nasal mucosa. Glands were tubular, varied in shape and size, and differed widely from those in the nose (Figs. 18 to 21). The most striking were long tubular glands, 1 to 8 mm in length, arising from the middle or distal part of the polyp. (Tos and Mogensen 1977).

These glands grew toward the stalk, parallel to each other and parallel to the longitudinal axis of the polyp (Fig. 18). Some were very simple narrow tubes; others had prominences, small round alveolar bulges, or small tubules that at times had undergone dichotomous division. (Tos 1982). (Figs. 19, 20and 21).

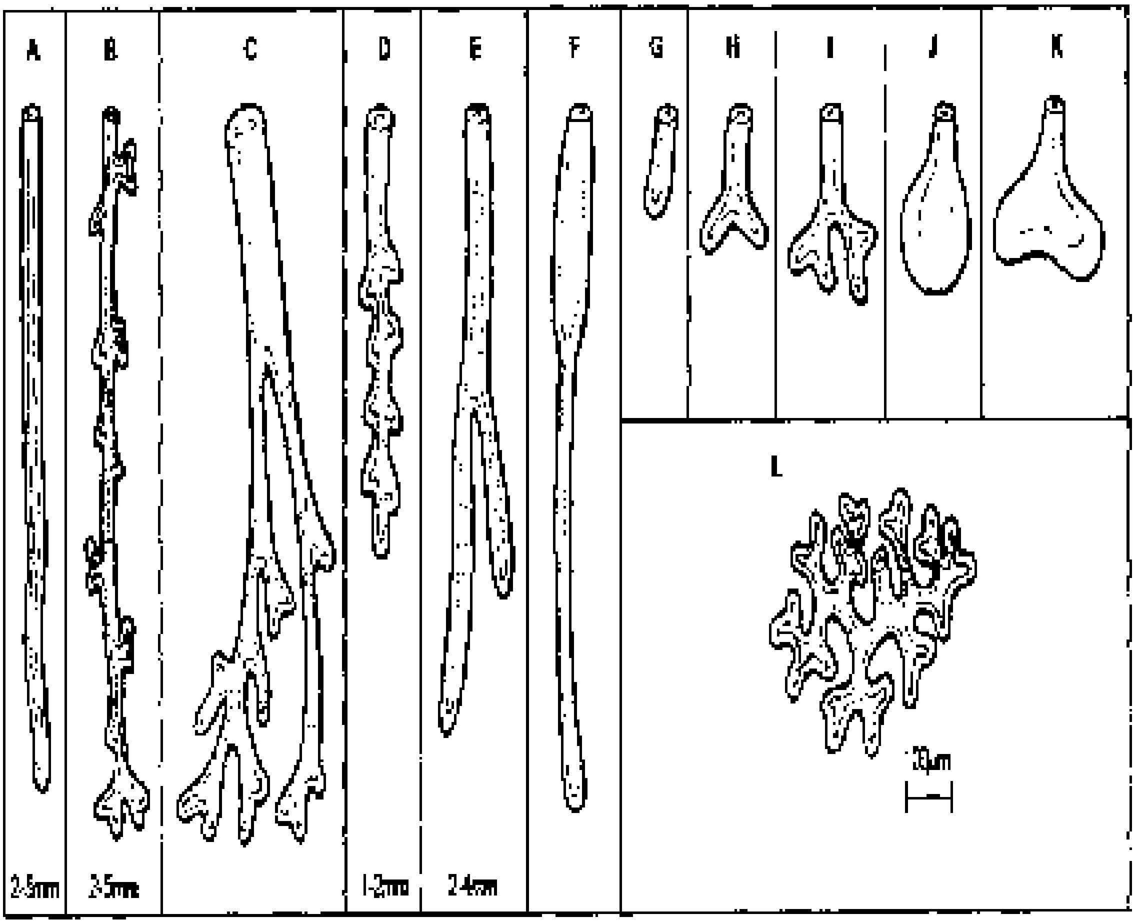


Fig. 18:Various shapes of mucous glands. A-F: Long tubular; G-I: Small tubular; J-K : Small cystic; L: Tubuloalveolar glands.Quoted from **Tos 1982.**



Fig. 19: Several long tubular glands in nasal polyp. PAS-alcian blue whole mount (original magnification *15). **Tos 1982**

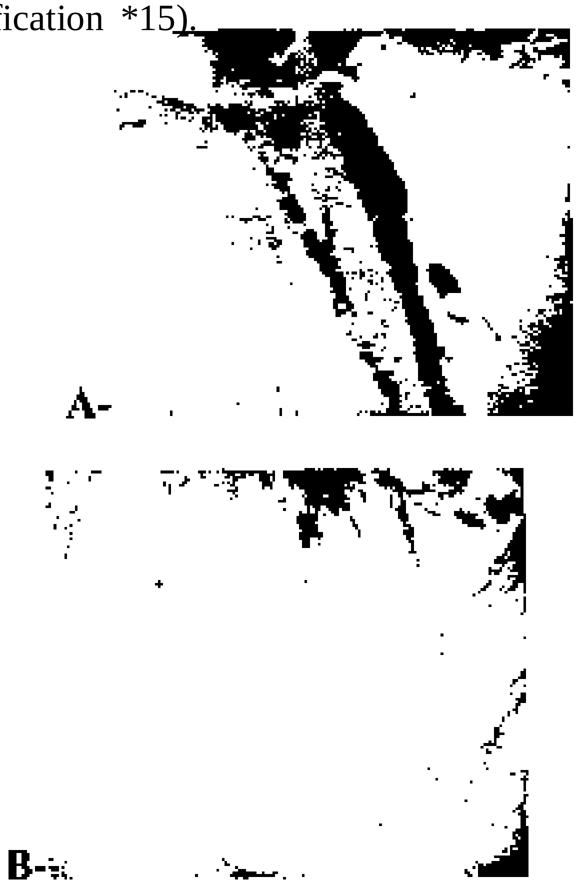
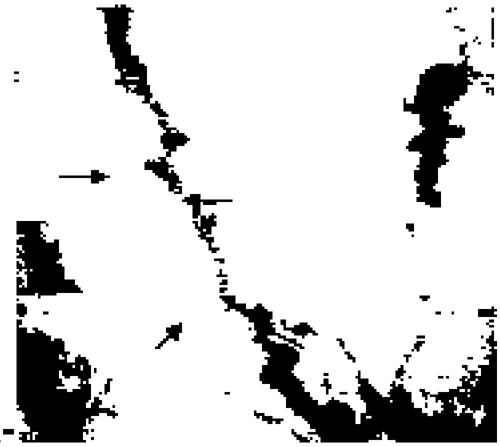


Fig.20 long tubular glands in nasal polyp at higher magnification. Quoted from Tos 1982.

A. Two active straight tubulous glands, (original magnification*25)

B. A long tubule with side bulges. PAS-alcian blue whole mount. (original magnification *25)



A-



B-

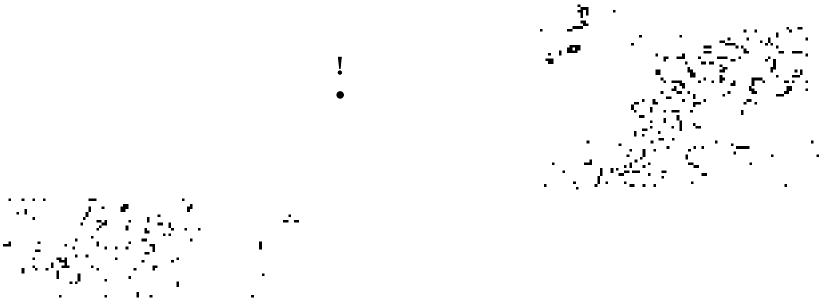


Fig.21: Example of branching with long tubular glands in nasal polyps.Quoted from Tos 1982.

A, The dichotomous division is just indicated imitating a screwdriver shape.
B, Branched long gland.PAS-alcian blue whole mount (original magnification x30).
C, A section along a branched tubular gland. Hematoxylin and eosin stain (original magnification xI00),

iU Cellular infiltrate:

A variety of inflammatory cells and mediators have been described in nasal polyps. Eosinophils, neutrophils, lymphocytes and their subgroups, plasma cells, mast cells, and macrophages have all been found in nasal polyps. Often, these cells are ' cated in the subepithelial, perivascular, and periglandular gions. **(Linder, et. al.,1993) (Fig. 22,23).**

The type of inflammatory cells and mediators may have important implications in the pathogenesis of nasal polyps.
terestingly, Ogawa in 1988 demonstrated an increase in the percentages of polyps infiltrated by eosinophils from 42% collected during the period 1975 to 1976 to 65% collected uetween 1983 to 1984 in Japan. The increase of polyps infiltrated with eosinophils supports the theory of immunoglobin E (IgE)-mediated hypersensitivity being an important etiologic factor of eosinophilic polyposis. **(Ogawa 1986).**

In polyps from patients with chronic rhinitis, neutrophils were found in large numbers, and some were closely attached to the basal membrane. Neutrophils migrate through the epithelium on the polyp surface by way of the gap in the basal membrane.
(Takasaka et al., 1986).

Distribution of degranulated and nondegranulated mast cells varies within polyps **(Fig. 24).** Degranulated mast cells occurred more commonly in the deep stroma than near the surface. The pedicle of the nasal polyp has more mast cells and more degranulated mast cells than the apex, making the stalk more important in the etiopathogenesis of the polyps. Degranulation of mast cells thus plays an important role in the formation of nasal polyps. **(Saseki 1986).**



Fig.22: Nasal polyp with highly edematous stoma infi-ltrated by neutrophils localized mainly subepithelialy, perigl-andularly (G), and periva-scularly. Hematoxylin and eosin stain section (Original magnification* 100).Quoted from Linder, et al.,1993

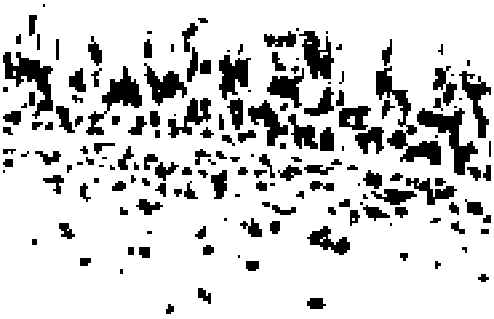


Fig.23: Migration of eosinophils (arrows) through the epithelium of a nasal polyp. The eosinophils are concentrated mainly beneath the basal membrane. Hematoxylin and eosin stain section (Original magnification x 100). Quoted from Linder, et al.,1993.

&



Fig.24: Granulated mast cell (arrow) and some neutrophils in the edematous

stroma of a nasal polyp with scattered fibroblasts Hematoxylin and eosin stain section (Original magnific-ation^{x}1000).Quoted from Saseki 1986.

II-Overview of basic medical mycology;

More than ever, fungal diseases have emerged as major challenges for physicians, clinical microbiologists, and basic scientists. The incidence of mycotic infections and the number and **diversity of pathogenic fungi have increased dramatically in recent years. Immunocompetent as well as immunocompromised individuals are at risk. One obvious indication of the growing breadth, complexity, and significance of mycological diseases is the need for an in-depth treatment of fungal rhinosinusitis, a disease that is increasing in incidence and is often difficult to diagnose and manage. On definition of fungal rhinosinusitis, the immune response of the host shapes the fungal manifestation which can be defined as invasive (acute, granulomatous, chronic), noninvasive (fungus balls, saprophytic), and allergic. There may exist overlap among individual cases. Prognosis and therapy depend on the particular fungal manifestation. (Eloy et al.,1997).**

The overview of basic medical mycology including the useful terminology, morphology and relevant laboratory methods for the diagnosis of some fungal genera and species implicated in fungal rhinosinusitis will be discussed through the following chapter (Table 4).

BB

Category	**Genera**
Zygomycetes	*Absidia* *Cunninghamella* *Mucor* *Rhizomucor* *Rhizopus*
Hyaline moulds	*Aspergillus* *Blastomyces Dermatidis* *Chryosporium* *Fusarium* *Paecilomyces* *Scedosporium* *Scopulariopsis*
Dematiaceous moulds	*Altemaria* *Bipolaris* *Cladosporium* *Curvularia* *Exserohilum*
Ascomycetous yeasts	*Candida*
Basidiomycetes	*Corpinus* *Cryptococcus neoformans Schzophyllum* *Ustilago*

Table 4. Some fungal genera and species implicated in fungal rhinosinusitis, quoted from **Eloy et al.,1997.**

1- Biology of fungi;

Fungi grow as *yeasts* and *moulds* (see glossary of mycologic terms). Moulds are characterized by the production of multicellular, filamentous colonies composed of branching cylindrical tubules that vary in diameter from 2 to 10 pm and re termed *hyphae.* The mass of intertwined hyphae that ^cumulates during active growth in vitro is a *mycelium.* Some hyphae are divided into cells by crosswalls, or *septa,* that typically are formed at regular intervals. One class of medically important moulds, the zygomycetes, which includes the agents C mucormycosis, produces hyphae that rarely are septated. Hyphae that penetrate the supporting medium and absorb nutrients are the vegetative or substrate hyphae. In contrast, aerial hyphae project above the surface of the mycelium and usually bear the reproductive structures of the mould. Under standardized growth conditions in the laboratory, moulds produce colonies with characteristic features, such as rate of growth, texture, and pigmentation. The genus, if not species, of most clinical mould isolates can be determined by microscopic examination of the ontogeny and morphology of their asexual reproductive spores or conidia (Table 5) (Beneke and Rogers,1996).

Yeasts are single cells, usually spherical to ellipsoidal in shape varying in diameter from 3 to 15 pm. Most yeasts reproduce by budding. Under certain growth conditions, some pathogenic yeasts produce buds that characteristically fail to detach and become elongated; continuation of the budding process then produces a chain of elongated yeast cells called *pseudohyphae* (Beneke and Rogers,1996).

Yeast colonies are usually soft, opaque, 1 to 3 mm in size and cream-colored. Because the colonies and microscopic morphology of many yeasts are quite similar, yeast species are identified on the basis of physiologic tests and a few key morphologic features. Some species of fungi are dimorphic and capable of growth as a yeast or a mould, depending on the environmental conditions (Beneke and Rogers,1996).

All fungi have an essential, rigid cell wall that determines the shape of the fungus. Cell walls are composed largely of carbohydrate layers and long chains of polysaccharides, as well as glycoproteins and lipid. During infection, fungal cell walls have important pathobiologic

properties. The surface components of the cell wall mediate attachment of the fungus to host cells. Cell wall polysaccharides may activate the alternative pathway of the complement cascade to stimulate an inflammatory reaction. Fungal cell wall material is degraded poorly by the host and can be detected with special stains. Cell walls release immunodominant antigens that may elicit cellular immune responses and diagnostic antibodies. Most fungal allergens are cell wall polysaccharides and glycoproteins. Some yeasts and moulds have melanized cell walls, imparting a brown or black pigment, and are described as dematiaceous. In several studies, melanin has been associated with virulence (Hogan et al.,1996).

In addition to their vegetative growth as yeasts or moulds, fungi can produce spores to enhance their survival. Spores can be readily airborne and dispersed, are more resistant to adverse conditions, and can germinate when conditions for growth are favorable. Spores can derive from asexual or sexual reproduction, which are called the anamorphic and teleomorphic states, respectively. Asexual spores are mitotic progeny and genetically identical. The medical fungi produce two major types of asexual spores, *conidia,* **and in the zygomycetes,** *sporangiospores* **(see glossary of mycologic terms).**

Informative features of spores include their ontogeny (some moulds produce complex conidiogenic structures) and morphology (size, shape, texture, color, and uni- or multicellular nature). In some fungi, vegetative cells may transform into conidia, producing thallic conidia (e.g., arthroconidia, chlamydospores). In others, conidia are produced by a onidiogenous cell, such as a phialide, which itself may be tached to a specialized hypha called a conidiophore. In the zygomycetes, sporangiospores result from mitotic replication and spore production within a sac-like structure called a sporangium, which is supported by a sporangiophore.(Larone 995).

^.-Taxonomy:

The ever-changing classification of fungi is fundamentally based on the process of sexual reproduction, which involves, in most cases, strains that can mate or undergo plasmogamy, nuclear fusion, meiosis, and the exchange of genetic information. The major taxonomic groups are the zygomycetes, ascomycetes, basidiomycetes, and deuteromycetes (or imperfect, asexual fungi).

In the zygomycetes, sexual reproduction results in a zygospore; vegetative hyphae are sparsely septate, and asexual reproduction occurs through sporangia. Representative genera are *Rhizopus, Absidia, Mucor,* **and** *Cunninghamella.* **With the ascomycetes, sexual reproduction involves a sac or ascus in which karyogamy and meiosis occur, producing meiospores called** *ascospores.* **Asexual reproduction is through mitospores called conidia, and moulds in this group have septate hyphae. Examples of ascomycetes include** *Ajellomyces* **(anamorphic genera,** *Blastomyces, Histoplasma), Arthroderma* **(anamorphic genera,** *Microsporum, Trichophyton),* **and yeast genera, such as** *Saccharomyces* **and** *Hansenula.* **In basidiomycetes, sexual reproduction results in four progeny basidiospores supported by a club-shaped basidium. The supporting hyphae have complex septa. These higher fungi include the mushrooms, as well as** *Filobasidiella neoformans,* **the teleomorph of** *Cryptococcus neoformans.* **The sexual spores associated with each of these groups are defined in the Glossary. (Si-Germain and SummerbeU** *,1996).*

The deuteromycetes are an artificial grouping of the imperfect or mitosporic fungi for which a teleomorph or sexual reproductive cycle has not been discovered. The anamorphic state is characterized by asexual conidia. When a sexual cycle is discovered, a species is reclassified to appropriately reflect its phylogeny. Familiar examples of deuteromycetes are *Aspergillus fumigatus, Candida albicans,* **and** *Coccidioides immitis.* **A species may be recognized and defined on the basis of its asexual (imperfect or anamorphic) state, but its teleomorph, or sexual identity, may have a different name. (Sutton et al.,1998).**

3-Concept of fungal disease?

Fungi most commonly cause disease by initiating an infection, which entails a portal of entry (for exogenous fungi), attachment to host cells or tissues, and the capacity to grow within the host, which implies the abilities to replicate at 37°C, obtain adequate nutrients, and evade the natural and specific host defenses. For the dimorphic pathogens, survival in the host requires an initial morphologic conversion to a tissue form of growth. For example, following their inhalation, the conidia or hyphal elements of C. *immitis, Histoplasma capsulatum,* **and** *Paracoccidioides brasiliensis* **convert to yeast cells (Campbell et al.,1996).**

Some fungi are capable of colonizing the surfaces of epithelial tissues without penetrating and invading below the surface. Many cases of fungal rhinosinusitis are characterized by

colonization rather than invasion (i.e., noninvasive disease). These fungi may elicit profound inflammatory and immune responses. Tissue invasion by fungi also stimulates the host defenses, which ultimately may kill or contain the fungal cells (Horner et al.,1995).

A few fungi produce potent toxins and mutagens known to ause serious human disease after exposure or ingestion. With the possible exception of certain species and strains of *Aspergillus* **and** *Fusarium,* **the best characterized toxigenic fungi are probably not agents of rhinosinusitis. Less potent irritants ad enzymes, however, may be elaborated by fungi that attack the local host cells and lead to physiologic or inflammatory damage, or immunopathology. Undoubtedly, such mechanisms contribute to certain cases of fungal rhinosinusitis. Another mechanism of disease production in fungal rhinosinusitis is induced by fungal cell wall antigens that stimulate allergic fesponses in the host (Panda et al.,1998).**

Rhinosinusitis follows inhalation of fungal spores into the nasal passages. Occasionally, however, the fungal cells can invade from a preexisting infection in the lung or elsewhere in the body. The outcome of inhaling an inoculum of spores depends upon several factors: (1) the number of spores inhaled; (2) the size of the fungal particles, which influences the depth to which they penetrate the nasal or respiratory passages; (3) the integrity of the nonspecific and specific host defenses; and (4) the pathobiologic potential or virulence of the particular fungus. Certain risk factors are well associated with specific mycotic infections, such as atopy with allergic fungal sinusitis, or diabetic ketoacidosis with rhinocerebral mucormycosis. (Ferguson et al.,1988).

Perhaps most cases of fungal rhinosinusitis, as with many other mycoses, occur in individuals who are predisposed to the disease because of compromised host defenses. A number of conditions and treatment modalities are recognized to increase susceptibility to mycotic infection. In general, people with impaired cell-mediated immunity are more susceptible to mycotic infection. This condition may be congenital or induced by other diseases, such as AIDS or hematogenous dyscrasias,

or by the administration of cytotoxic or immunosuppressive drugs to manage cancer or transplantation. For example, many cases of candidiasis, cryptococcosis, and aspergillosis are associated with recent treatment with corticosteroids. The condition of the host innate defenses and immunity may dictate whether an individual at risk develops invasive, noninvasive, or allergic forms of fungal rhinosinusitis. (Murphy,1996).

Taxa	Colony appearance	Microbiology
Altemaria Alternate	Powdery to velvet to cottony; grey to dark green-black.	Chains of brown, clavate conidia (20— 65 x 10—20 pm) with transverse and longitudinal septa.
Aspergillus Fumigatus	Powdery to velvety texture; blue- green.	Long conidiophores produce terminal vesicles with phialides that bear chains of small conidia (2.5—3.5 pm); phialides are on the upper portion of the vesicle only.
Bipolaris hawaiiensis	Rapid growth; velvety texture; gre^-black. ■9	Ceniculate conidiophores sympodially produce large, ellipsoidal brown thick- walled macroconidia with rounded ends (15—35 x 5—10 pm), ***4***—6 septa.
Curvularia Lunata	Flat! cottony to velfety texture; dark brown to black.	Geniculate conidiophores sympodially produce curved conidia (20—35 x 10— 15 pm) with usually 3 septa; one of the middle cells is usually larger, darker, and asymmetrical.
Exserohilum Rostratum	Rapid growth; velvety texture; grey-black.	Geniculate conidiophores sympodially produce large, generally elongate, brown thick-walled macroconidia (30—125 x 10—25 pm), 7—9 septa; conidia have a protruding dark basal scar at the hilum.
Rhizopus Arrhizus	Rapid growth; abundant cottony aerial mycelium; pale grey with black sporogonia.	Broad sparsely septate hyphae, brownish rhizoids; sporangiophores terminate in spherical black sporangia (50—250 pm) with prominent columellae; many sporangiospores (6—8 pm).
Scedosporium Apiospermum	Cottony, white to dark grey.	Single or branched anneilides, sometimes on conidiophores forming synnemata, produce ellipsoidal, yellow to light brown conidia (5—15 ***X*** 3—6 pm).

Table 5. Prominent features of common agents of fungal Rhinosinusitis, quoted from **Beneke and** Rogers,**1996.**

Ill-Diagnosis of mycotic infection

In the diagnosis of fungal infections, the following laboratory strategies may be employed: (1) microscopic examination of fresh clinical specimens or histopathologic preparations; (2) culture of the etiologic agent from clinical material; (3) serology and skin testing; (4) radiographic techniques; and in the future, (5) polymerase chain reaction (PCR) methods to detect specific fungal DNA in clinical specimens. The most common and definitive methods are the direct microscopic examination and culture, and whenever possible, both approaches should be used (Larone,1995).

Specimens

Similar to respiratory specimens, the sterility of the paranasal sinuses depends^upon the anatomic location and method of collection. Abscesses and exudates from sequestered lesions often contain abundant infectious material. Any purulent drainage should be collected, although it is likely to be contaminated and polymicrobic. When examining patients with sinusitis, any exudate, infected material, or necrotic tissue should be rapidly transported to the laboratory for direct examination and culture. Specimens should be collected aseptically and transported in closed sterile containers. Swabs should be avoided. Extensive surgical debridement should be avoided on immunocompromised patients who are neutropenic or thrombocytopenic.In the laboratory, processing often depends on the volume and consistency of the sinus specimen, which may be mucoid, purulent, bloody, or necrotic, and range from slightly viscous to gelatinous. If biopsied tissue was obtained, it can be minced with sterile scissors and divided for culture, direct examination, and fixation for histopathologic staining. (Chandler,1998).

1- Microscopic examination;

Specimens can be examined directly by light microscopy. The

specimen is mixed with 10% or 20% potassium hydroxide (KOH) and examined. The slide should contain only a small mount of material, and the preparation should be thin to discern mgal structures. The ideal stain for the direct examination is a mixture of KOH and calcofluor white, which requires a microscope with ultraviolet illumination at the appropriate wavelength. Staining with calcofluor white markedly increases ie sensitivity. Calcofluor white stains fungal cell wall polysaccharides nonspecifically, which fluoresce under the impropriate optics. (flg:25). Artifacts include cotton fibers and similar material. Because neither K6H alone or combined with calcofluorwhite stains fungi undervlsible light, the cell walls of dematiaceous fungi appear light - 91 dark-brown and can be detected withou| difficulty. Fungal sells also can be seen with a Jram stain, although they stain variably, and the stain may mask any inherent pigmentation (Larone4995). >

Exudates as well as tissue call be stained with routine histopathologic preparations, such as hematoxylin and eosin (H&E), Gomori •methenamine silverl(GMS), or periodic acid— Schiff (FA'S)." The GMS and PAS Stain are superior to H&E stains, and the fungal cell walls stain black or red, respectively. The melanin of dematiaceous fungal walls often is apparent on H&E slides. The Fontana-Masson melanin stain may be useful in differentiating the dematiaceous fungi from nonmelanin containing hyphae such as *Aspergiilu. (Friedman etal.,1991)*

In examining microscopic preparations of specimens, valuable information can be gleaned from careful observation of the appearance of the fungal elements. The size, morphology, and quantity of any fungal cells should be noted, as well as whether the cells possess melanin and are darkly pigmented. Fungi may present as yeast cells, hyphae, or combinations of

these structures. The presence of sclerotic bodies or muriform cells, which are dematiaceous spherical cells with transverse septations, about 5 to 20 pm in diameter, is indicative of chromomycosis. With hyphae, the uniformity of width, branching, and septations should be noted. If fungal spores are present, distinctive features, such as their size, shape, surface texture, and number of cells may permit the identification of heir genus. Sometimes, more than one species is involved. The majority of fungal agents of rhinosinusitis present as hyphae. (Larone ,1995).



Fig. 25: Fluoresent optical brighteners bind to the fungal cell wail and provide a highly sensitive and rapid method for microscopic examination of the specimens.(KOH-calcofluor white stain, original magnification x200) quoted from Larone,1995.

2- Culture identification:

Most fungi occur in nature and grow readily on simple sources of nitrogen and carbohydrate. In the laboratory, the traditional medium is Sabouraud's agar, which contains glucose and beef extract (pH 5.0); it has been used because it does not jpport readily the growth of bacteria. The morphologic haracteristics of fungi used for identification have been described from growth on Sabouraud's agar at 30°C. Other media, however, such as inhibitory mould agar, have improved the recovery of fungi from clinical specimens. For the recovery of edical fungi from nonsterile specimens, antibacterial antibiotics (e.g., gentamicin, chloramphenicol) and cy- Hoheximide are added to the media to inhibit bacteria and saprophytic moulds, respectively. Most agents of fungal rhinbsinusitis are moulds. The most common agents are species of *Aspergillus* **and dematiaceous hyphomycetes (see Table-4). Their identification is based on the morphology of their conidia, which are produced in vitro. If the isolate is sporulating, the genus usually can be determined readily. The identification at the species level often requires detailed observation of the conidia, or with zygomycetes, the sporangiospores. Speciation is based on differences in the shape, size, and texture of the conidia and conidiogenous structures. Occasionally, physiologic differences are useful. There are several useful manuals and taxonomic keys to guide the mycologist through the identification of these moulds (Campbell et al.,1996).**

Some moulds are difficult to identify because they fail to sporulate, are polymorphic, or produce reproductive structures that are atypical. The identity of a few problematic moulds can be clarified by the detection of species-specific ^Aoantigens(Pasarell et al.,1990).

With others, DNA-based methods are being developed to identify various taxa, as well as individual strains. Most PCR methods

have exploited variable regions of the ribosomal DNA to detect fungal taxa and strains. Other approaches have used polymorphisms in other genes, specific oligonucleotide probes, and the technique of random amplified polymorphic DNA with single PCR primers. Methods have been developed for *Aspergillus* **and other hyaline and dematiaceous moulds, (taylor ,1999).**





Fig. 26: Altemaria x 100. Fig. 27: Aspergillus x 100. Qouted from Larone,199S Qouted from Larone,1995





Fig. 28: Curvularia x 100. Fig. 29: Bipolaris x 100.
Qouted from Larone,1995 Qouted from Larone,1995

3- Histology laboratories:

Histologic techniques are more time-consuming and routinely require one or more days for results to become available, but they provide the only means of determining whether a fungus is invading into host tissue. They have the further advantages of producing permanent preparations that can be viewed at any time, and of allowing differential staining methods that can suggest particular etiologies. The routine histologic stain is the hematoxylin and eosin (H&E) stain, a combination of basic and acidic dyes that stains nuclei blue or dark purple, and extracellular and cytoplasmic material pink. It is extremely useful for studying human tissue's reaction to infection, but like the Gram stain it usually does not highlight the fungal cell wall, and as a result is not likely to detect fungal cells that are present in small numbers. In cases where fungal cells are numerous and can be seen on H&E preparations, the stain still tends to be ineffective because poor differentiation of the fungus from surrounding host material makes further interpretation difficult. Notable exceptions where the H&E stain can be useful are for the hyphae of *Rhizo pus* **and** *Aspergillus* **species, and** *Scedosporium apiosperinum,* **which sometimes stain well, and the cells of some of the dematiaceous species, which may have a brown color that is not obscured by the H&E stain (Fig. 30)(Chandler,1998).**

Several special stains are used routinely for fungi. The silver stain employs deposition of silver onto the fungal cell wall, and because it can detect even a single cell it undoubtedly is the most sensitive of the commonly used histologic stains (Fig.31). No histologic specimen should be considered to be negative for fungus unless a silver stain has been performed. The periodic acid-Schiff stain perhaps is a more specific stain and works well in many cases, but it is less sensitive than silver because dead or metabolically senescent cells may not be stained. Its advantage is that it does not stain so darkly as to

hinder the study of fungal morphology (e.g., septation) as the silver stain sometimes does. Both of these stains, however, can mask the natural color of fungal cells, especially if the color is faint. Examination for the intrinsic brown color of dematiaceous fungi must include the use of H&E or unstained preparations as needed (Chandler and Watts, 1987).

The Fontana-Masson (FM) stain for melanin is a differential stain that can be very useful when a mould is discovered in tissue. Several of the most common agents of fungal sinusitis are dematiaceous in culture but typically are 'aline (colorless) in tissue. The FM stain stains these hyphae brown to black. A positive FM result can allow more narrow diagnosis of cause, and may influence the choice of antifungal -uemotherapy. Caution in using the FM stain is advised, however, there appears to be variation in FM staining results among isolates of *Aspergillus* fu*migatus, Aspergillus flavus,* and *Fusarium solani.* Also, in the case of *Aspergillus niger*, the .egetative hyphae are FM-negative, but the conidia and the specialized hypha that bears the conidia are FM-positive.Al- though some uncertainties about FM staining for moulds need to be resolved, the stain still can be useful if careful study of fungal morphology is integrated (Kimura and McGinnis,1998).

Currently, the Grocott methanamine silver stain (GMS) staining technique is the gold standard and most common method used to detect fungal organisms. It takes advantage of the polysaccharides present in the fungal cell wall to create a contrasting image defining the organism from the background. Unfortunately, this technique is not necessarily specific for fungi. Furthermore, it is not as effective in creating a contrast in mucin specimens that are known to contain high concentrations of polysaccharides due to greater background staining. (Ponikau, 1999).

A novel staining method for the detection of fungal organisms in human tissue was recently developed (Fungalase- F; Anomeric, Inc, Baton Rouge, LA). Chitin, a homopolymer of P (1,4)- linked A-acetylglucosamine, composes the cell wall of most fungi but is not found in mammals. Natural chitin sources other than fungi include the exoskeletons of crustaceans and insects. The recombinant chitinase used in the Anomeric Fungalase kits is an enzyme cloned from *Vibrio parahemolyticus* that binds tightly and specifically to chitin. When directly labeled with fluorescein, it can be used as a histochemical probe and marker, in an assay where catalytic activity is not a significant factor, to visualize fungal cell walls in various specimens. Fluorescein-labeled chitinase binds with sufficient avidity to fungal cell walls to survive repeated washings and has been shown to be an effective diagnostic tool in detecting invasive fungal organisms in human tissues.(Taylor, 2002).(fig. 32).



Fig. 30: Fungal hyphae scattered through out the mucin(H&E stain,original magnification -100). Qouted from Larone,1995

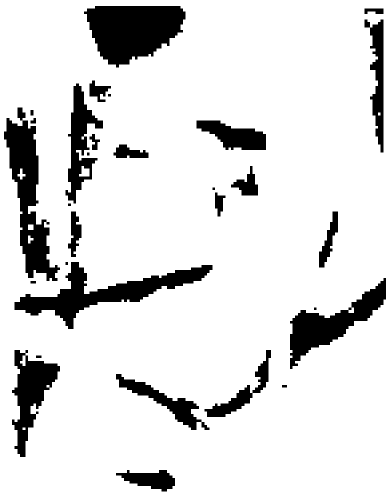


Fig. 31: Fungal hyphae scattered through out the mucin (Silver stain,original magnification><400). Qouted from Larone.1995

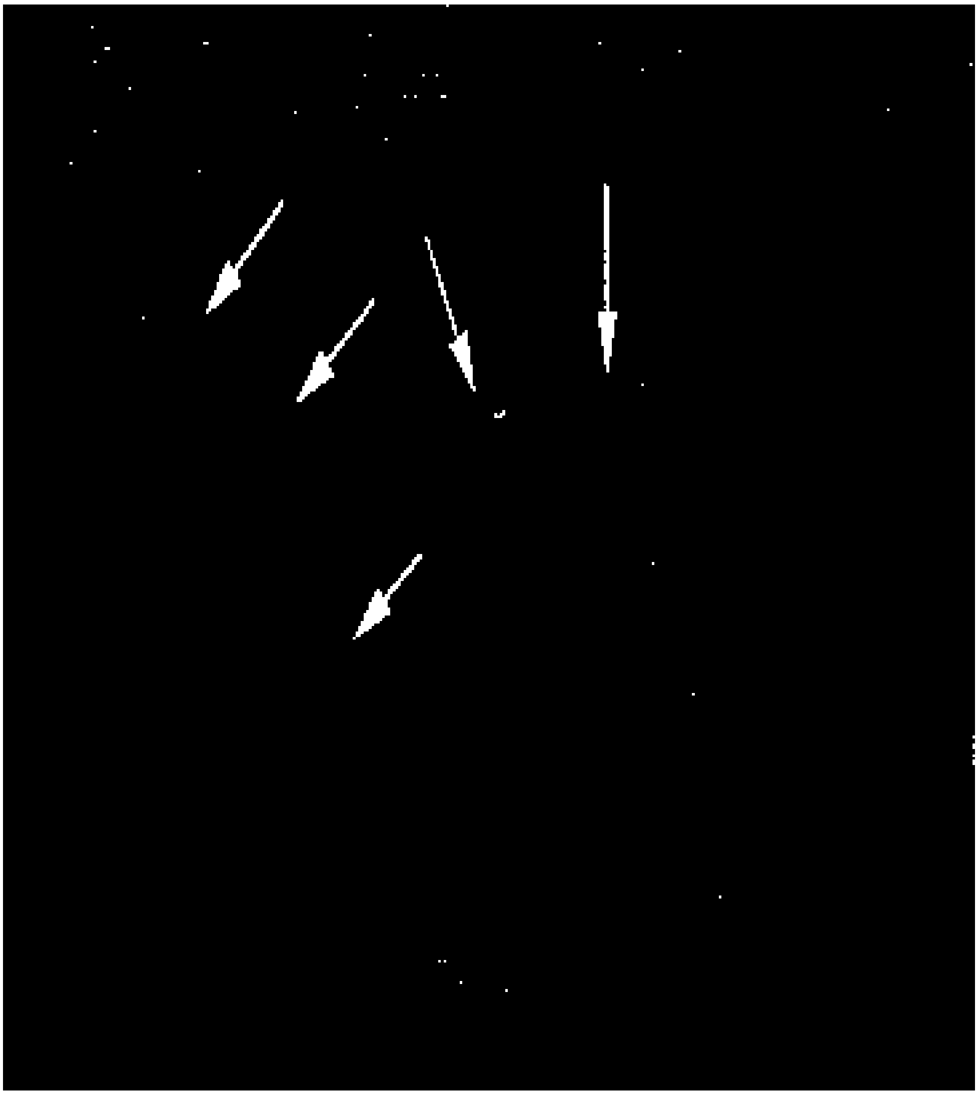


Fig. 32: Chitinase staining of a serial section of allergic mucin reveals the fungal organisms in cross section (3 are marked by white arrows) and longitudinal section (yellow arrows) within the cluster of eosinophils. Quoted from Taylor, 2002

4- Serology and Skits Tests

Most patients with allergic fungal rhinosinusitis have circulating antibodies to the antigens and allergens of the offending mould. Precipitins and specific IgE and IgG antibodies can be detected in serum by radioimmunoassays and enzyme immunoassays. (Mabry et al.,1998)

The antibody class and skin test results can help to differentiate among invasive, noninvasive, and allergic fungal oinusitis. Both immediate and delayed skin tests can be performed. Commercial antigens are available for some of the igents of fungal rhinosinusitis but unavailable for others, most notably the *Bipolaris* species, a major cause of allergic fungal rhinosinusitis. Antigens, however, can be extracted readily from the fungal growth of a patient's isolate. (Schubert and Goetz,1998).

However, a group of patients exists with the clinical features of allergic fungal rhinosinusitis with positive cultures from there eosinopilic fungal mucin but with no systemic evidence of fungal allergy , locally produced fungal-specific IgE might explain the pathogenesis of this condition because evidence is emerging that this local mechanism is involved in some patients with chronic rhinosinusitis with polyposis these patients may represent another diagnostic group , suggesting that the so called allergic eosinophilic fungal mucin might not always arise from a type 1 immune response . as corollary to this it should be expected that patients who have systemic fungal-specific IgE would have local IgE in the sinus mucin if this condition were truly IgE mediated (Collins et al.,2004).

Since current models used to study nasal fungi have several limitations, attempts have been made to create a new model to study the Allergic Fungal Sinusitis (AFS) that targeted specific areas of the nasal cavity and could accurately predict even small amounts of fungi, polymerase chain reaction had ' een used previously to test for a variety of pathogens in the nasal mucosa and liingr(Rahaadaft|et ai., 1997).

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The sensitivity of PCR is high, detecting as little as one >py of DNA, also, PCR can be used to quantify the amount of fungi present; the fungal burden as determined by PCR may be a better correlate of the severity of,.clinical symptoms associated with AFS. Lastly, using protected swabs, specific subsites of the nasal mucosa can be tested for the presence and quantity of fungi. Knowing the quantity of fungus present in the sinonasal cavity of patients With ICRS and comparing it with normal control subjects would help elucidate amy mechanism by which threshold quqntaties of fungus could lead to sinonasal disease. Further more, correlating clinical severity with fungal load could further define its role in the pathogenesis of CRS. (Schuller et aL, 2004)

Catten et al. recently described the use of polymerase chain reaction (PCR) techniques to test for the presence of fungal elements within the nasal cavity. In this study, the samples were collected using a cytology brush along the septum and inferior turbinate. Using a universal primer, 10 (40%) of the 25 patients with CRS and 18 (42%) of the 42 normal controls tested positive for the presence of fungal DNA by PCR. (Catten et al., 2001).

Accumulation of allergic fungal mucin eventually leads to the increasingly well-recognized radiographic findings character- ristic of AFS. Heterogeneous areas of signal intensity within paranasal sinuses filled with allergic fungal mucin frequently are identified,'on CT scans.ATffi'ougErthese findings are not specific for AFS, they remain relatively "charicteristl: of the disease and may provide preoperative information" supportive of a diagnosis of AFS. This characteristic, which is best identified using soft- tissue algorithms on CT scan, has been fthe focus of some interest (Manning et al., 1997). -[3] *i*

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The hemosiderin occurring within inspissated mucin is responsible for th| areas of increased signaj intensity. This was disputed by -Zinreich et al, however they wefe unable to identify increased hdinosiderin within typical allergic fungal mucin. Current evidence points to the presence of accumulations of heavy metals (eg, iron, manganese) and calcium salt precipitation within inspissated allergic fungal mucin as the most likely causes of these radiographic findings. (Zinreich et al., 1988).

Expansion, remodeling, or thinning of involved sinus walls is common in AFS and is thought to be caused by the expansile nature of the accumulating mucin. Areas of high attenuation are found within the expanded paranasal sinuses in all patients. Similar radiographic findings can be caused by rare osteoid/chondroid matrix-producing sinonasal sarcomas or meningiomas. Bony erosion of the sinus walls and extension into adjacent cavities has been mentioned in many reports, usually focusing on intracranial extension. In most series, a rate of approximately 20% bony erosion with extension into surrounding vital cavities is reported. A statistically significant association was identified between expansion of paranasal sinuses involved with disease and the presence of bone erosion. The ethmoid sinus was the most commonly involved sinus, while the adjacent lamina papyracea was the most common bone to exhibit demineralization. Extension of AFS beyond the confines of the paranasal sinuses most commonly occurred into the orbit, followed by the anterior, middle, and

posterior cranial fossae, respectively. (Lund et al., 2000). (fig. 33)

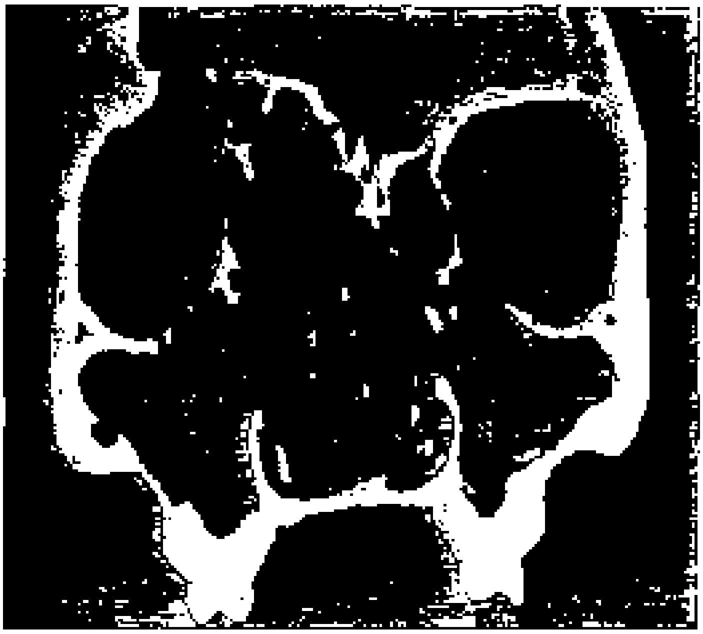


Fig. 33: Coronal CT scan of a patient, showing extensive allergic fungal sinusitis involving the right side with mucocele above the right orbit and expansion of the sinuses on the right. Quoted from **Lund** et **al., 2000**

A staging method that relies on CT appearance to define the extent of disease in CRS patients is by no means perfect because of the problems of interpretation posed by previous surgery, the occasional absence of the frontal sinus, and the difficulty in differentiating between opacification resulting from inspissated mucus and mucosal inflammation, which may have prognostic significance. Not withstanding theses criticism, CT scanning does offer one of the few assessments of rhinosinusitis that can be objectively quantified and can be applied to the vast majority of patients in routine clinical practice. The staging system that was proposed by Kennedy (fig. 34) which was based on the assessment of the extent of the disease dividing the

gross changes through out the sinuses into four stages has been used for some years in the quantification of inflammatory disease before surgical intervention^ Lund, 1997).

<u>Staging system proposed by Kennedy</u>

Stage 0 Normal
Stage I Anatomic abnormalities
All unilateral sinus disease Bilateral disease limited to ethmoidal sinuses Stage II Bilateral ethmoidal disease with involvement of one dependent sinus.
Sage ffl Bilateral ethmoidal disease with involvement of two or more dependent sinuses.
Stage IV Diffuse sinonasal polyposis. '

Fig. 34: CT staging system for rhin^sinusitis proposed by Kennedy. Quoted from Kennedy DW. Prognostic factors, outcomes and staging in ethmoid sinus surgery, 1992. |

B-Magnetic resonance imaging: :

MRI also can provide information useful in preoperative identification of allergic fungal mucin, but it usually is not necessary when making the diagnosis unless the disease has extended into the intracranial cavity or confusion exists with the diagnosis. Som et al. have pointed out that protein concentrations exceeding 28% cause a, decreased signal on Tl- and T2-weighted MRI images becausp; of protein cross-linking and slower macromolecular motion'.' This effect is more pronounced on T2-weighted images because of prolonged magnetic field relaxation times. The high protein and low water concentration of allergic fungal mucin, coupled with the high water content within surrounding edematous paranasal sinus mucosa, gives rise to rather specific MRI characteristics^ Som et al. 1990) (fig. 35)

In a series of 10 patients with AFS, Manning et al., 1997 demonstrated that hypointense central T1 signal, central T2 signal void, and the presence of increased peripheral T1/T2 enhancement were highly specific for AFS when compared to other forms of fungal sinusitis (invasive fungal sinusitis, fungal ball) and mucocele. On MRI, the hypointense signal looks black and can be mistaken for absence of disease in the paranasal sinuses because a black signal in the sinuses on CT scan indicates the absence of disease. The combined CT scan and MRI findings provided a radiographic appearance that was "righly specific for AFS.

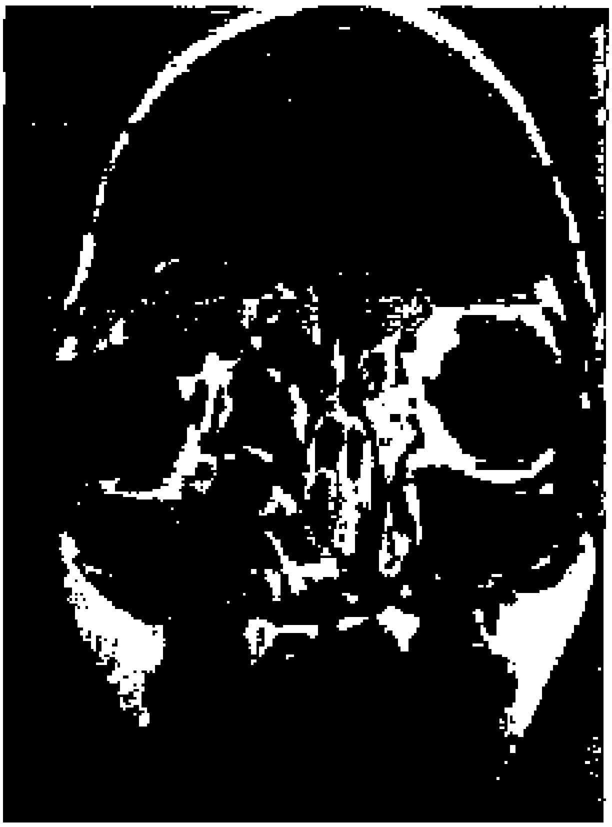


Fig. 35: Coronal MRI of the same patient, showing expansion of the sinuses with allergic mucin and polypoid disease; the hypointense black areas in the nasal cavities are the actual fungal elements and debris. The density above the right eye is the mucocele. The fungal elements and allergic mucin in allergic fungal sinusitis always look hypointense on MRI scanning and can be mistaken for absence of disease. Quoted from **Lund** et **al., 2000**

MATERIAL AND METHODS

MATERIAL AND METHODS

(1) Patients and Controls:

A-Inclusion criteria:

This study was conducted on one hundred (100) consecutive patients with the clinical diagnosis of chronic rhinosinusitis (CRS) with bilateral nasal Polyposis.

Fifty (50) Volunteers with no history of nasal or paranasal sinus disease, with no symptoms of inhalant allergy, no history of smoking and with normal appearing mucosa confirmed by nasal endoscopy, served as a control group.

"Patients and controls were instructed not to use any steroidal medications 1 month prior to the study to prevent diminishing of the eosinophilic inflammation.

B-Exclusion criteria:

All patients with history of diabetes mellitus, local or systemic corticosteroids medication 1 month before the study were excluded from this study.

All patients studied presented to the Kasr El Aini outpatient clinic during the period of the last two years starting from August 2002 to July 2004. Their ages ranged between fourteen to fifty six years. The study included sixty six males and thirty four females, that were subjected to:

a Full ENT examination.

□ Nasal endoscopic examination.

□ Computed tomographic scanning of the Nose and paranasal sinuses (coronal and axial 5mm thick cuts) in both soft tissue and bone windows with enhacement.

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(2) Collection and culture technique of nasal lavage:

The collection, handling and culturing techniques were carried out using the novel method described by **ponikau et al., 1999.** They found that this method allows collecting as much aucus as possible improving the fungal yield in the cultures:

Two puffs of phenylephrine hydrochloride 1% were sprayed into each nostril to produce vasoconstriction. The spray also icreases the nasal lumen and consequently the yield from nasal lavage. After approximately 2 minutes each nostril is flushed ! ith 20 mL of sterile saline using a sterile syringe with a sterile curved blunt needle. The patient takes a deep inspiratory breath and holds it before the injection of saline. The patient then forcefully exhales through the nose during the flushing. The "'turn is collected in a sterile pan.

The collected fluid was placed into centrifuge tubes and sent directly to the mycology laboratory where the specimen is processed under a laminar flow hood to prevent contamination. One vial (10 mL) of sterile dithiothreitol (Sputolysin; Oxoid, England) was diluted with 90 mL of sterile water. The collected specimen is suspended with an equal volume of diluted dithiothreitol and vortexed for 30 seconds. The mixture was allowed to stand at room temperature for 15 minutes while the dithiothreitol breaks apart the disulfide bonds, thus liquefying the mucus allowing the fungal elements to be released from the viscid mucus and contact the growth media adequate for fungal growth.

The mixture is then centrifuged at 3000 *rpm* in a 50-mL tube for 10 minutes. The supernatant was discarded, and the sediment was vortexed for 30 seconds. One-half milliliter of the prepared sediment was inoculated onto each of three types of cultures: 1- Sabouraud Dextrose agar plate, 2- Sabouraud Dextrose agar plate containing gentamicin (5 pg/mL), and "hloramphenicol (15 pg/mL) and 3-inhibitory mould agar ontaining 5% chloramphenicol (15 pg/mL) and cycloheximide (5 mg/mL). The plates were incubated at 30°C and allowed to grow for 30 days. The plates were examined at 2-day intervals, and all cultures were identified. The resulting

growth was sub iltured on Com meal agar for proper identification and isolation of the different species harvested.

(3) Collection of surgical specimens and histologic examination:

The patients were subjected to endoscopic sinus surgery (ESS) under general anesthesia.

The principle of maximum mucus preservation was adhered to during the acquisition of surgical specimens. This enabled the assigned pathologist to find allergic mucin and fungal elements within the mucus. All the surgical procedures were performed without a power microdebrider to ensure maximal mucin collection. In addition, use of suction devices was limited. The mucus was manually removed together with inflamed tissue and placed on a saline- moistened nonstick sheet. Specimens were not placed directly on a surgical towel or on gauze because these carriers absorb a large amount of the mucus.

The paraffin-embedded blocks were cut into several serial sections of 5 nun each and baked onto positively charged glass slides. The sections were stained with hematoxylin and eosin (H&E), Gomori Methenamine Silver (GMS), Periodic Acid- Schiff stain (PAS) and the remaining sections were available for chitinase staining and negative controls.

Each of the hematoxylin and eosin, the GMS and the PAS- stained specimens were thoroughly examined under light microscopy for the presence of firngal elements. A specimen was considered positive if at least one fungal hyphae could be clearly identified in an entire slide.

The serial section slides immediately adjacent to the PAS slides were used for chitinase staining following the technique stated by **Taylor et al.,2002.** The specimens were deparaffinized and rehydrated using xylene, ethanol, and distilled water. Because the eosinophil-rich environment increases the background staining of fluorescein, the sections were incubated for 30 minutes in 1% chromotrope 2R to decrease nonspecific binding. The specimens were then washed in phosphate buffered saline (PBS) for 15 minutes and incubated in 5% periodic acid for 10 minutes. The slides were then rinsed with distilled water and incubated in 5% bovine serum albumin containing 0.1% azide for 10 minutes. One drop of the fluorescein- labeled chitinase stain (Fungalase-F; Anomeric, Inc, Baton Rouge, LA) was then added, and the slides were incubated in a dark, humidified chamber for 30 minutes. Once the staining was completed, the slides were rinsed and washed in PBS solution for 2 minutes. The specimens were then mounted with 1 drop of Vectashield mounting medium, and a coverslip was applied. All stained specimens were stored in the darkness at 4 °C.

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Each slide was then examined with a Zeiss microscope equipped with Zeiss UV FL vertical illumination for epifluorescence and a fluorescein filter system (Carl Zeiss, Inc, Oberkochen, Germany) (magnification range xlOO, x200 , x400 andxlOOO) within 1 week to avoid fading. Fluorescence photomicrographs were obtained through both the immunofluorescence microscope and the confocal laser microscope.

A specimen was considered positive if all of the 3 following criteria were met: (1) the element in question enhanced brightly,

(2) it had the characteristic morphology of fungal hyphae, and

(3) it traveled through the mucin indicating the tubular structure, not merely overlying the specimen. Because chitinase binds to the fungal cell wall only, specimens were examined for the fluorescent outline of fungal hyphae.

These outlines vary in shape, ranging from circular to oblique to longitudinal depending on the angle at which the specimen was cut. The outlined rims of the fungal hyphae could be followed as it traveled through the full thickness of the specimen, ensuring that the slides were not secondarily contaminated by environmental fungi.

RESULTS

RESULTS

In this study allergic fungal rhinosinusitis (AFS) was common among adolescents and young adults, with a mean age of diagnosis of **29.9** years with a range (17 - 60) and standard deviation 9.271 for the whole 100 patients. The average age of the male predominance in this series was **27.65** years among a total of 66 males with a range (17-55) and standard deviation 8.447, while the average age of the female predominance was **36.04** years among a total of 34 females with a range (19 - 60) and standard deviation 9.787. The male-to- female ratio appeared to be age dependent The younger the average age, the more likely to have a male predominance. However, this predominance was almost equal when all ages were evaluated collectively. **(Table 6) (Fig. 36)**

Sex	Number	Minimum age	Maximum age	Mean age	Std.Deviation
[redacted]					

Table 6: Descriptive statistics of the age among the paients.

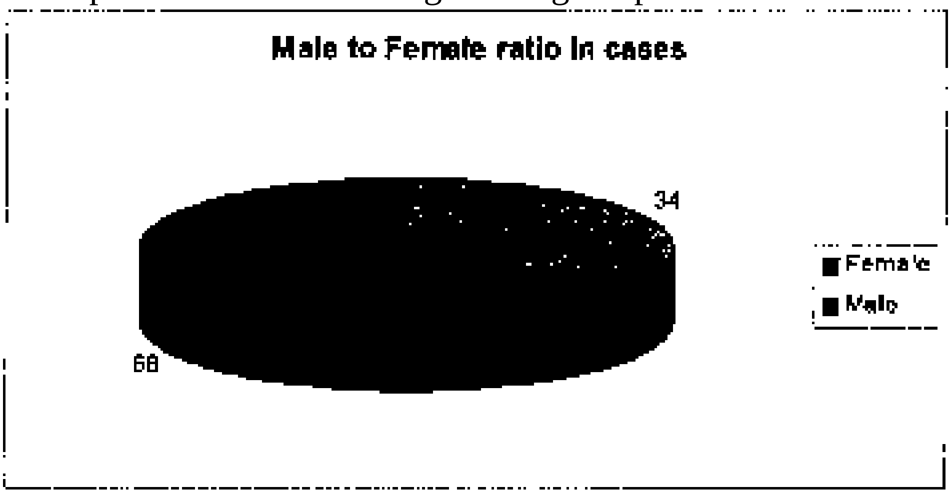


Fig. 36: The male to female ratio among patients.

The average period of the complaint was 1.2 years, 58 patients did not have any previous surgical intervention, 22 patients had a single previous nasal operation to relief symptoms within two years, 15 patients had dual operations within two years, and 5 patients had 3 or more operations within the same period.

All patients had a broad spectrum of inflammatory mucosal thickening ranging from minimal polypoid changes (N=32) to massive polyposis (N=68), most patients had eosinophilic mucin within their presenting polypoidal disease (N=92).(Fig. 37 to 42).



Fig. 37: diffuse polyps in the left anterior nasal cavity of a female patient having chronic rhinosinusitis (CRS) with bilateral polyposis.



Fig. 38: Thick visid eosinophilic mucin in the nasal vestibule of the same patient, when removed there was diffuse polyps of various sizes.





Fig. 40: Thick visid mucin and crustations in middle meatus(small arrows).

Fig. 39: Thick eosinophilic mucin in the middle meatus (F) of a patient having CRS with polyposis.

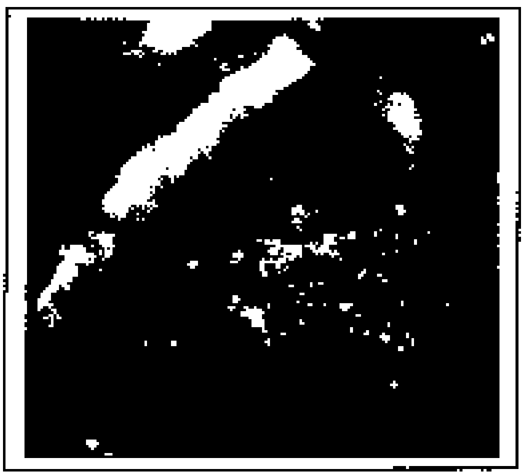




Fig. 42: Fungal debris during its removal from ethmoids of the same patient in fig.41.

Fig. 41: Fungal mass in the right maxillary sinus of a

patient having CRS with bilateral polyposis.

<u>I- Mvcological results:</u>

The new collection and culturing method stated by **Ponikau et** aL, 1999 that was used in this study **(Figure** 46& 47) resulted in cultures positive for fungus in 100 **(100%)** of 100 consecutive patients (N-100) with CRS using the Sabaroud Dextrose Agar (SDA) medium and in 50(100%) of the control group of normal, healthy volunteers using the same medium (N=50). **(Table** 7).

The cultures using the SDA, Chloramphenichol and Gentamycin medium resulted in 98 (98%) positive cultures of the same 100 consecutive patients and 49 (98%) positive cultures of the control group of normal healthy volunteers using the same medium. **(Table 8).**

The cultures using the SDA and Cyclohexamide medium resulted in 83 (83%) positive cultures of the same 100 consecutive patients and 41 (82%) positive cultures of the control group of normal, healthy volunteers using the same medium. **(Table 9).**



Fig. 43: Showing thick allergic mucin sucked from the region of the ethmoid bulla in a patient having CRS with bilateral polyposis.

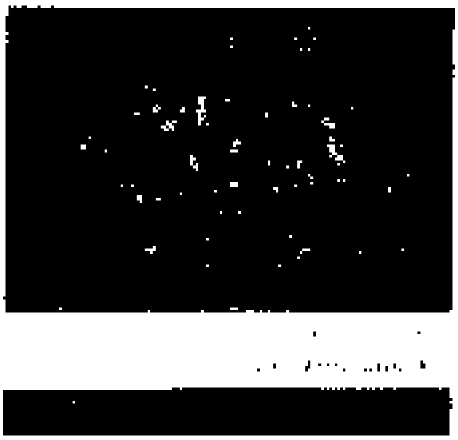


Fig. 44: Allergic mucin, fungal debris and polyps shown after being removed from a patient having CRS with bilateral polyposis.(scale in cm.)

A total of 281 positive cultures were yielded using the three types of culture media, with an average of 2.8 organisms per patient and a maximum of 5 different organisms per patient. A total of 29 different genera or species of fungi were identified (Table **10, 12,** [1]3,**14** and **15).** Interestingly, in the control group of normal, healthy volunteers **(N=50)** a total of 140 fungus-positive cultures grew using the three culture media, with an average of 2.8 different organisms per volunteer, and a maximum of 4 different organisms per subject Table **11** and **12,13, 14** and **15).** The organisms grown from the "ontrols were not markedly different than those from the CRS patients. **(Figs. 45** and **46).**

Aspergillus niger was the most common organism encountered (51%)followed by *Altemaria species* (28%), *Aspergillus fumigatus* (28%), *Pseudoallescheria boydii* (20%), *Aspergillus flavus* (17%) *Penicillium species* (14%) and *Aspergillus versicolor(* 10%) when 7DA was used as the culture medium for the nasal wash of the patients in this study.

Aspergillus niger was the most common organism encountered (50%)followed by *Altemaria species* (28%), *Aspergillus fumigatus* (28%), *Pseudoallescheria boydii* (18%), *Aspergillus flavus* (15%), *Penicillium species* (10%) and *Aspergillus versicolori* 10%) when S.D.A., chloramphenicol and gentamycin was used as the culture medium for the nasal wash of the patients in this study.

Aspergillus niger was the most common organism encountered (46%)followed by *Altemaria species* (23%), *Pseudoallescheria boydii* (15%), *Aspergillus fumigatus* (14%), *Aspergillus versicolor{*6%) and *Penicillium species* (5%) when S.D.A. and cyclohexamide was used as the culture medium for the nasal wash of the patients in this study.

Acremonium	1	1	4	8
Altenaria	28	28	32	64
A.Niaer	51	51	25	50
A. Fumigates	28	28	14	28
AFIavus	17	17	9	18
A. Versicolor	10	10	6	12
A. Terrus	2	2	1	2
A.Niduians	4	4	2	4
Aspergillus Spp.	2	2	2	4
Aureobasidum	1	1	1	2
Bipolaris	5	5	4	8
Cabdida albicans	6	6	1	2
Chaetomium	1	1	1	2
Chryosporium	4	4	4	8
Ciadosporium	8	8	1	2
Curvularia	8	8	4	8
Dactviaria	4	4	0	0
Dematlaceous Funaus	1	1	0	0
Epicocum	3	3	1	2
Fusarium	4	4	0	0
Mucor	5	5	0	0
Nlgrosporia	2	2	0	0
Rhizopus	1	1	0	0
Peniciliium	14	14	5	10
Pithomvces	4	4	2	4
Pseudoallescheria bovdii	20	20	1	2
Stachybotrys	9	9	0	0
Trichoderma	4	4	1	2
Trichosporon	1	1	1	2

Table 7: Number of fungi isolated from the patients (n=100) and controls (n=50) using the SDA medium.

p ....:» .... * .— Fungu*			iSPiifVNBBSHHI	
Acremonium	3	3	3	6
Altenaria	28	28	28	56
A Niger	50	50	24	48
A. Fumioatus	28	28	13	26
AFIavus	15	15	8	16
A Versicolor	10	10	6	12
ATerrus	2	2	1	2
ANidulans	3	3	2	4
Aspergillus Spp.	2	2	2	4
Aureobasidum	1	1	1	2
Bipolarls	3	3	3	6
Cabdida albicans	5	5	0	0
Cbaetomium	1	1	1	2
Chryosporium	4	4	4	8
Cladosporium	8	8	1	2
Cun/ularia	7	7	3	6
Dactyl aria	4	4	1	2
Dematiaceous Fungus	1	1	1	2
Epicocum	2	2	2	4
Fusarium	3	3	1	2
Mucor	3	3	1	2
Niprosporia	1	1	1	2
Rhizopus	0	0	1	2
Penlcilllum	10	10	2	4
Pithomyces	4	4	2	4
Pseudoallescheria boydii	18	18	1	2
Stachybotrys	8	8	0	0
Trichoderma	4	4	1	2
Trichosporon	0 I	0	1	2

Table 8: Number of fungi isolated from the patients (n=100) and controls (n=50) using the SDA, Chloramphenicol and Gentamycin medium.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Acremonlum	3	3	3	6
Attenaria	23	23	25	50
A. Nioer	46	46	24	48
A.Fumipatus	14	14	7	14
A. Flavus	4	4	2	4
A. Versicolor	6	6	5	10
ATerrus	0	0	0	0
ANidulans	0	0	0	0
Aspergillus Spp.	0	0	0	0
Aureobasldum	0	0	1	2
Bipolaris	2	2	2	4
Cabdlda albicans	0	0	1	2
Cbaetomium	0	0	0	0
Chryosporium	0	0	1	2
Cladosporium	0	0	0	0
Curwlaria	3	3	2	4
Dactvlaria	2	2	1	' 2
Dematiaceous Fungus	0	0	0	0
Eplcocum	0	0	0	0
Fusarium	0	0	1	2
Mucor	1	1	1	2
Niprosporia	0	0	1	2
Rhlzopus	0	0	0	0
Penicllllum	5	5	0	0
Pithomyces	0	0	1	2
Pseudoallescheria bovdii	15	15	1	2
Stachybotrvs	0	0	1	2
Trichoderma	0	0	1	2
Trichosporon	0	0	1	2

Table 9: Number of fungi isolated from the patients (n=100) and controls (n=50) using the SDA and Cyclohexamide medium.

[redacted]			
! *Acremonium*	1	3	3
utenaria	28	28	23
'.Niger	51	50	46
A.Fumioatus	28	28	14
A.FIavus	17	15	4
A. Versicolor	10	10	6
A Terms	2	2	0
A.Niduians	4	3	0
■*spemillus Spp.*	2	2	0
| *Aureobasidum*	1	1	0
1 *Bipolaris*	5	3	2
1 ʳ*abdlda albicans*	6	5	0
^*haetomium*	1	1	0
Chrvosporium	4	4	0
Ciadosporium	8	8	0
Curvuiaria	8	7	3
Dactyiaria	4	4	2
^ematiaceous Funpus	1	1	0
Epicocum	3	2	0
Fusarium	4	3	0
Mucor	5	3	1
Nigrosporia	2	1	0
Rhizopus	1	0	0
Penhillium	14	10	5
Pithomvces	4	4	0
Pseudoallescheria boydii	20	18	15
1 *Stachybotrys*	9	8	0
Trichoderma	4	4	0
Trichosporon	1	0	0

Table 10: Comparison of the frequency of isolation of fungi using the three culture media from the patients (n=100).

[redacted]			
Acremonium	4	3	3
Aitenaria	32	28	25
A Nicer	25	24	24
A Fumkjatus	14	13	7
AFlavus	9	8	2
A Versicolor	6	6	5
ATerrus	1	1	0
ANidulans	2	2	0
Aspemillus Spp.	2	2	0
Aureobasidum	1	1	1
Bipoiaris	4	3	2
Cabdida albicans	1	0	1
Chaetomium	1	1	0
Chrvosporium	4	4	1
Ciadosporium	1	1	0
Curvularia	4	3	2
Dactylaria	0	1	1
Dematiaceou8 Fundus	0	1	0
Epicocum	1	2	0
Fusarium	0	1	1
Mucor	0	1	1
Niarosporia	0	1	1
Rhfzopus	0	1	0
Peniciiiium	5	2	0
Pithomyces	2	2	1
Pseudoallescheria boydii	1	1	1
Stachvbotrvs	0	0	1
Trichoderma	1	1	1
Trichosporon	1	1	1

Table 11: Comparison of the frequency of isolation of fungi using the three culture media from the controls (n=50).

	Patient?	Controls
Total number of positive cultures and (%)	281(93.7%)	140(93.3%)
Total number of negative cultures and (%)	9(3%)	10(6.6%)
Average number of organism per case.	2.8	2.8

Table 12: Comparison of the mycological results between the patients and the controls in this study.

Category of cases studied	No. of organisms	Frequency	Percent
Controls	0	0	0.00
	l	7	14.00
	2	19	38.00
	3	19	38.00
	4	5	10.00
	5	0	0.00
	Total	50	100.00
Patients	0	0	0.00
	1	15	15.00
	2	34	34.00
	3	38	38.00
	4	12	12.00
	5	1	1.00
	Total	100	100.00

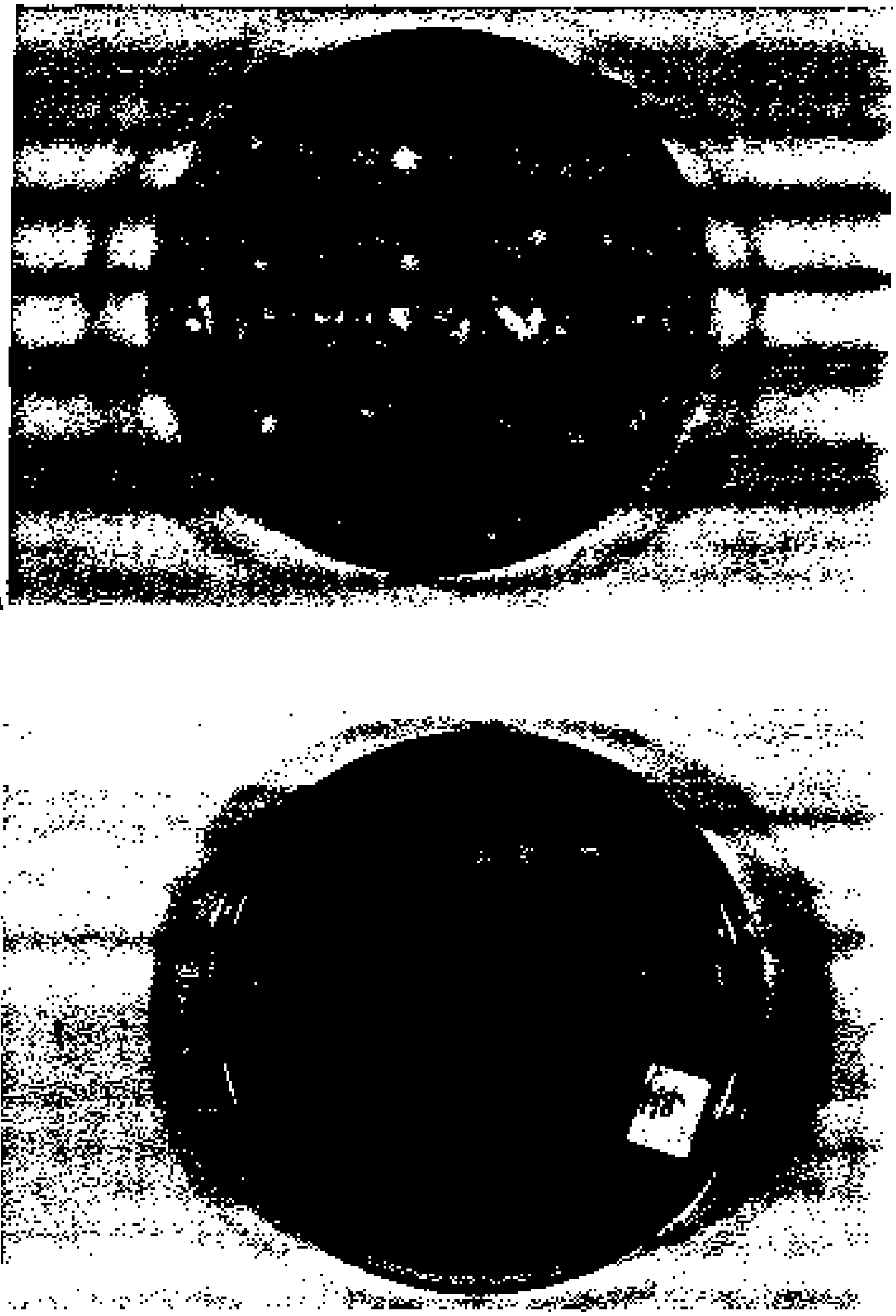
Table 13: The frequency of organisms recovered from patients and controls using the S.D.A. medium.

м* m Mm «		[ff&quencT	Percent
Controls	0	1	2.00
	1	11	22.00
	2	21	42.00
	3	14	28.00
	4	3	6.00
	5	0	0.00
	Total	50	100.00
Patients	0	2	2.00
	1	20	20.00
	2	39	39.00
	3	28	28.00
	4	9	9.00
	5	2	2.00
	Total	100	100.00

Table 14: The frequency of organisms recovered from cases and controls using the S.D.A., chloramphenicol and gentamycin medium.

Category of cases	I		
Controls	0	9	18.00
	1	15	30.00
	2	21	42.00
	3	3	6.00
	4	2	4.00
	5	0	0.00
	Total	50	100.00
Patients	0	17	17.00
	1	38	38.00
	2	30	30.00
	3	9	9.00
	4	5	5.00
	5	1	1.00
	Total	100	100.00

Table 15: The frequency of organisms recovered from patients and controls using the S.D.A. and Cyclohexamide medium.



A B

Fig. 45: Pure culture of *Stachybotrys species:* A- Surface.
B- Reverse.



A



B



C

Fig. 46: The microscopic morphology of fungi encountered in patients and controls in this present study (40 x objective).

A. Curvularia species.

B. Altemaria species.

C. Stachybotrys species.

Statistical analysis

1 - Statistical analysis of the number of negative cultures:

A. The number of negative cultures in patients according to the culture medium used:

i- Sabaroud Dextrose Agar.

The cultures results in all of the 100 consecutive patients was 100% positive. There was no negative cultures.

ii- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The number of negative cultures in patients was 2. Compared statistically to the results encountered in the Sabaroud Dextrose Agar. The P.value was 0.031 i.e. :(significant).

iii- Sabaroud Dextrose Agar and Cyclohexamide.

The number of negative cultures in patients was 17. Compared statistically to the results encountered in the Sabaroud Dextrose Agar. P.value = 0.000 i.e.: (highly significant).

B. The number of negative cultures in controls according to the culture medium used:

i- Sabaroud Dextrose Agar.

The cultures results in all of the 50 controls was 100% positive. There was

no negative cultures.

ii- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The number of negative cultures in controls was 1. Compared statistically to the results encountered in the Sabaroud Dextrose Agar. The P.value was 0.002 i.e: (significant).

iii- Sabaroud Dextrose Agar and Cyclohexamide.

The number of negative cultures in patients was 9. Compared statistically to the results encountered in the Sabaroud Dextrose Agar. P.value = 0.000 i.e.: (highly significant).

C. Comparison of the number of negative cultures in patients versus controls according to the culture medium used:

i- Sabaroud Dextrose Agar.

The cultures were 100% positive in both patients and controls and no statistics were computed because S.D.A. as -ve / +ve is a constant.

ii- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical comparison of the negative cultures for both patients and controls resulted in a P.value 1.00 i.e.:(insignificant).

iii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical comparison of the negative cultures for both patients and controls resulted in a P.value 0.879 i.e.:(insignificant).

2- Statistical analysis of the average number of the organisms in the used media:

A. patients:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results using the Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin **(average=2.28)** compared to the results in patients of S.D.A. **(average=2.48)** resulted in a P.value was 0.002 i.e: (significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results using the Sabaroud Dextrose Agar and Cyclohexamide **(average=1.24)** compared to the results in patients of S.D.A. **(average=2.48)** resulted in a P.value = 0.000 i.e. :(highly significant).

B. Controls:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results **(average=2.3)** compared to the results in controls of S.D.A. and Cyclohexamide **(average=1.64)** resulted in a P.value = 0.000 (highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results using the Sabaroud Dextrose Agar and Cyclohexamide **(average=1.64)** compared to the results in patients of S.D.A. **(average=2.44)** resulted in a P.value = 0.000 i.e. :(highly

significant).

C. Average no. of organisms in patients versus controls according to the culture medium used:

i-Sabaroud Dextrose Agar.

ihe average number of the patients versus controls resulted in a P. value 0.001 (higly significant).

ii- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The average number of the patients versus controls resulted in a P. value 0.003 (higly significant).

ui- Sabaroud Dextrose Agar and Cyclohexamide.

The average number of the patients versus controls resulted in a P. value 1.000 (not significant).

3- Statistical analysis of the cultures results of the commonly encountered organisms:

*Aspergillus niger:

A. patients:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Aspergillus niger cultures in patients using S.D.A., chloramphenicol and Gentamycin media, compared to the Aspergillus niger cultures results in patients using S.D.A. resulted in a Rvalue = 0.000 (highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Aspergillus niger cultures in patients using S.D.A and Cyclohexamide media compared to the Aspergillus niger cultures results in patients using S.D.A. resulted in a P.value = 0.000 (highly significant).

B. Controls:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Aspergillus niger cultures in controls using S.D.A., chloramphenicol and Gentamycin media. Compared to the Aspergillus niger cultures results in controls using S.D.A. resulted in a Rvalue =0.000 (highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Aspergillus niger cult
in controls using S.D.A and Cyclohexamide media. Compared to
Aspergillus niger cultures results in controls using S.D.A. resulte
a P.value =0.000 (highly significant).

C. Statistical comparison between results in patients versus Controls:

*-Sabaroud Dextrose Agar.

The percentage of the patients versus control was 51.0% (patients) 50.0% (controls) with a P. value 0.908 (insignificant)

M- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The percentage of the patients versus control was 50.0% (patients) 8.0% (controls) with a P. value 0.817 (insignificant)

iii- Sabaroud Dextrose Agar and Cyclohexamide.

The percentage of the patients versus control was 46.0% (patients) 48.0% (controls) with a P. value 0.817 (insignificant)

**Alternaria species:*

A. Patients:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Altemaria species cultures in patients using S.D.A., chloramphenicol and Gentamycin media. Compared to the Altemaria species cultures results in patients using S.D.A. resulted in a P.value = 0.000 (highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Altemaria species cultures in patients using S.D.A and Cyclohexamide media compared to the Altemaria species cultures results in patients using S.D.A. resulted in a P.value = 0.000 (highly significant).

B. Controls:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Altemaria species cultures in controls using S.D.A. chloramphenicol and Gentamycin media, compared to the Altemaria species cultures results in controls using S.D.A. resulted in a P.value = 0.000 (highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Altemaria species cultures in controls using S.D.A and Cyclohexamide media. Compared to the Altemaria species cultures results in controls using S.D.A. resulted in a P.value =0.000 (highly significant).

C. Statistical comparison between results in patients versus Controls:

i- Sabaroud Dextrose Agar.

The percentage of the case versus control was 28.0% (patients) 64.0% (control) with a P. value 0.000 (highly significant)

ii- Sabaroud Dextrose Agar, Chloramphenicol and Gentamydn.

The percentage of the case versus control was 28.0% (patients) 56.0% (control) with a P. value 0.001 (highly significant)

iii- Sabaroud Dextrose Agar and Cyclohexamide.

The percentage of the case versus control was 23.0% (patients) 50.0% (control) with a P. value 0.001 (highly significant)

*Aspergillus fumigatus:

A. Patients:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Aspergillus fumigatus cultures in patients using S.D.A., chloramphenicol and Gentamycin media. Compared to the Aspergillus fumigatus cultures results in patients using S.D.A. resulted in a P.value - 0.000 i.e. :(highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Aspergillus fumigatus cultures in patients using S.D.A and Cyclohexamide media compared to the Aspergillus fumigatus cultures results in patients using S.D.A. resulted in a P.value = 0.000 (highly significant).

B. Controls:

i- Sabaroud Dextrose Agar, Chloramphenicol and Gentamycin.

The statistical analysis of the results of the Aspergillus fumigatus cultures in controls using S.D.A., chloramphenicol and Gentamycin media. Compared to the Aspergillus fumigatus cultures results in controls using S.D.A. resulted in a P.value = 0.000 i.e. :(highly significant).

ii- Sabaroud Dextrose Agar and Cyclohexamide.

The statistical analysis of the results of the Aspergillus fumigatus cultures in controls using S.D.A and Cyclohexamide media compared to the Aspergillus fumigatus cultures results in controls using S.D.A. resulted in a P.value =0.000 (highly significant).

C. Statistical comparison between results in patients versus controls:

i- Sabaroud Dextrose Agar.

The percentage of the patients versus controls was 28.0% (patients) 28.0% (controls) with a P. value 1.000 (insignificant)

>i-Sabaroud Dextrose Agar+ Chloramphenicol + Gentamycin.

Tie percentage of the patients versus controls was 26.0% (patients) 28.0% (controls) with a P. value 0.817 (insignificant)

h'i- Sabaroud Dextrose Agar+ Cyclohexamide.

The percentage of the patients versus controls was 14.0% (patients) 14.0% (controls) with a P. value 1.000 (insignificant)

II. Histopathological results:

Of the 100 patients operated upon , fungal elements (hyphae, destroyed hyphae, conidiae, and spores) were found in 100 histologic specimens (100%) (Fig. 47, 48 and 49). The allergic mucin, containing clusters (or sheets) of degenerating eosinophils and their by-products (Fig. 50), was found in 92 (92%) of 100 consecutive surgical cases. Interestingly, in the remaining 8 cases in which the allergic mucin was absent, the eosinophils were also almost completely absent in the harvested nasal mucosa and polyps. Two of these patients also had an acute bacterial onset with neutrophil predominance.

As expected with different species and various stages of hyphal maturity, there were a varity of sizes of fungal organisms present within the mucin. This size range was noted on GMS, PAS and Fungalase stains. Only 60 (60%) of the GMS and PAS stained specimens were positive for one or more fungal elements.

All specimens stained negatively on GMS staining were noted to have smaller and fewer hyphae as identified with the fungalase staining technique. Overall, although the GMS stained slides were positive for the identification of larger hyphae this staining method failed to detect many of the smaller hyphae within the same specimen that could be easily visualized with the fungalase stain. Furthermore, for some specimens, the higher concentration of polysaccharides within the mucin stained intensely with the GMS and PAS stain making identification of fungal elements more difficult. (Fig. 51,52 and 53).

At least one fungal element could be identified within the mucin of each of the 40 negative GMS-stained specimens using the Fungalase N=40(100%). Eosinophilic inflammation was observed within the mucin and tissues; however, firngal hyphae were identified only within the mucin but not within the tissues. In the majority of cases, multiple hyphae were present and could be identified in perpendicular oblique and longitudinal cross section. (Fig. 54).



Fig. 47 a & b: Two sections stained by Grocott methanamine silver stain (GMS) showing many fungal hyphae within the mucin from a case of CRS with bilateral polyposis (Silver stain, original magnification x400).

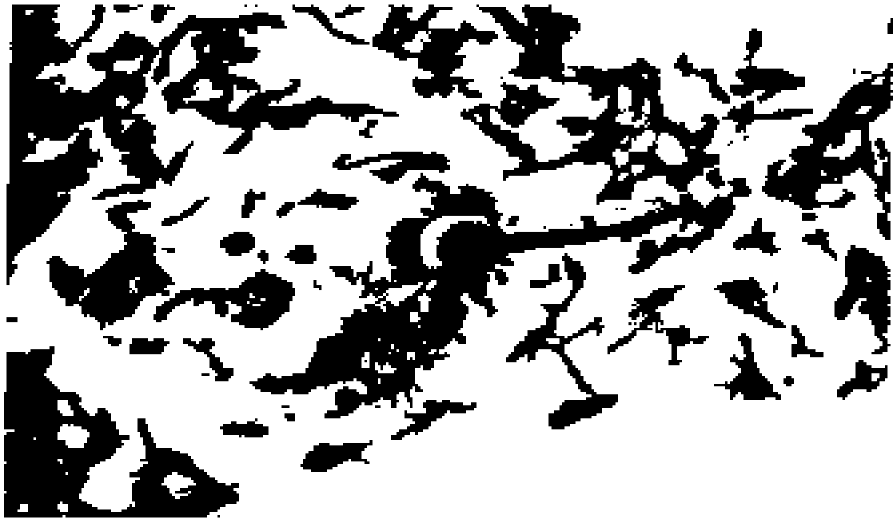


Fig. 48: Fungal hyphae detected within mucus removed from the nose showing the fruiting pattern characteristic of Aspergellus fungus from a case of CRS with bilateral polyposis. (PAS x 400)

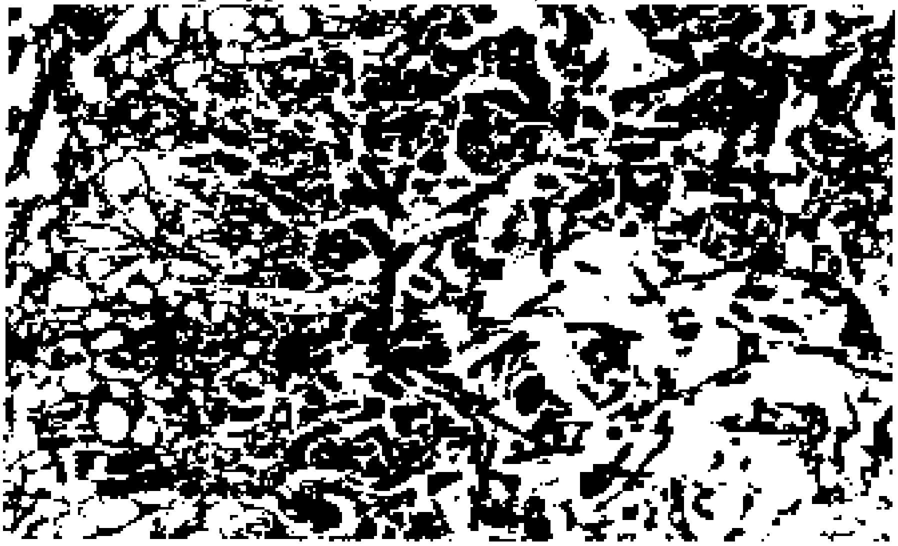


Fig. 49:.Some Candida fungal hyphae & spores detected within mucus of a case of CRS with bilateral polyposis (H & E x 400).

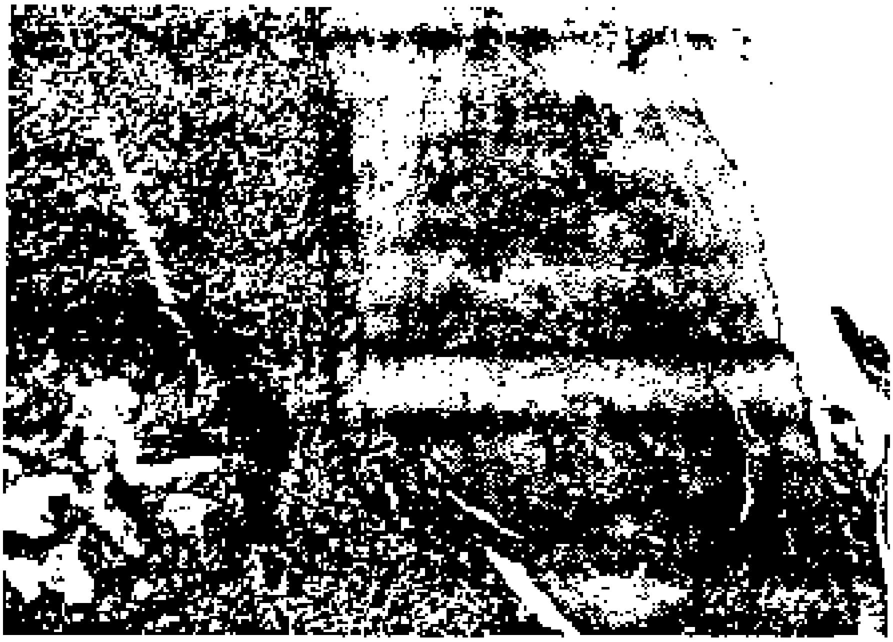


Fig. 50: A part of an inflamed allergic nasal polyp covered by mucous-secreting columnar epithelium with excess mucus on its surface containing clusters (or sheets) of degenerating eosinophils and their by-products. No fungal hyphae could be detected. (H&Ex 100).



Fig. 51: Showing nasal polyps from a patient of CRS with bilateral polyposis with excess mucus stained intensely with the GMS stain, making identification of fungal elements difficult. (Silver stain, original magnification x200).

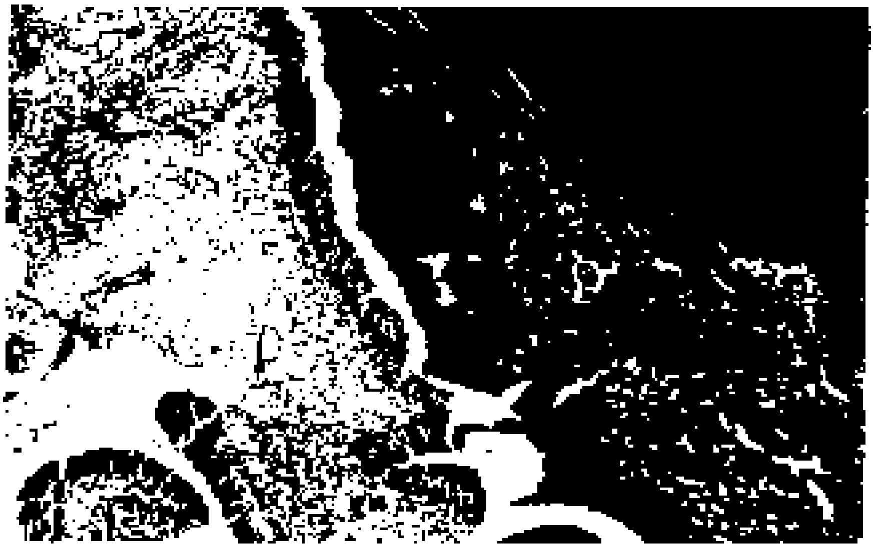


Fig. 52: The same field of the previous case stained by PAS stain. Also no fungal hyphae could be detected. (PAS x 200).

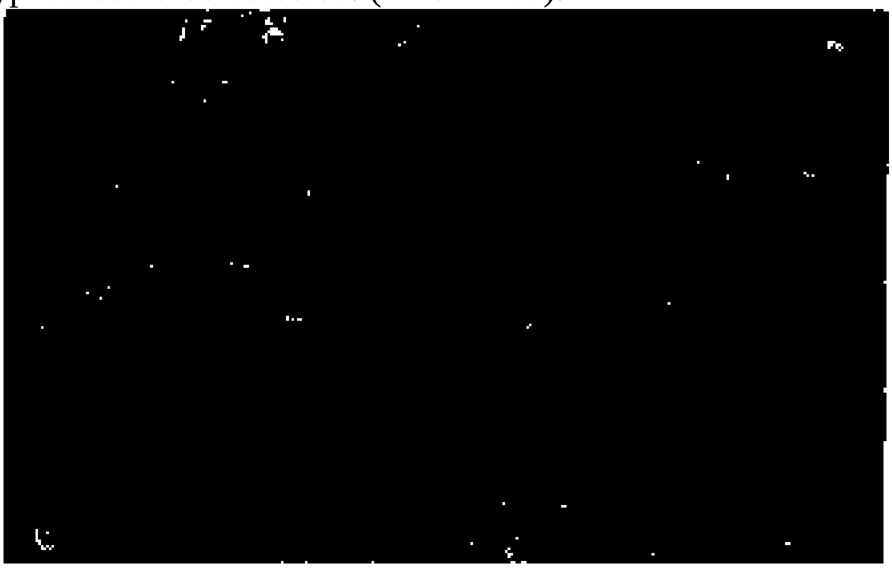


Fig. 53: Section from the previous case stained by fluorescent- labeled chitinase *(fungalase)* showed many fungal hyphae within the mucus that wasnot demonstrated using the GMS or PAS stains (Fungalase x 1000).



Figure 54: Sections stained by fluorescent-labeled chit- inase *(fungiase)* showing fungal hyphae which are few and small in size cutted in longitudinal and cross sections within the clusters of eosinophils , ensuring the high sensitivity of the stain. (FunglasexlOOO).

III- Radiological results:

1- In our routine radiologic studies, small pockets of thick mucin between the polypoid material inside the sinuses, thin layer of mucus coating the inflamed mucosa or high-attenuation areas, reflecting the allergic mucin (precisely where we have found it during surgery) were noted on CT scan in 23 (23%) of our patients of CRS. Of which :

a- 5 (5%) were bilateral ethmoidal disease with involvement of one dependent sinus (stage II- Kennedy classification)

b-18 (18%) were bilateral ethmoidal disease with involvement of two or more dependent sinuses on each side (stage HI- Kennedy classification) (Figure 55).

2- On the other hand, 77 (77%) of our patients of CRS had a CT scan image with the mucus filling the entire sinus with hyperintense and inhomogeneously opacified areas, massive polyposis and bone erosion.(stage IV- Kennedy classification) (Figure 56).



Fig. 55: CT scan of a patient with chronic rhinosiusitis showing moderate inflammation and thickening and typical small areas of

hyperattenuation (arrows) correlating with the intraoperative finding of eosinophilic(allergic) mucin.



Fig. 56: Coronal CT scan of a patient with bilateral allergic sinusitis with hyperintense areas and inhomogeneity of the sinus opacification; the hyperintense areas appear whitish in the center of allergic mucin. There is erosion of the sphenoid roof and extension of the disease into the anterior cranial fossa.

DISCUSSION

Discussion

The origin of chronic rhinosinusitis remains elusive. Recently the central role of fungi in this disease process has been recognized where fungal rhinosinusitis presents in five clinicopathologic forms, each with distinct diagnostic criteria, treatment and prognosis. The invasive forms are acute fulminant, chronic and granulomatous (indolent) invasive fungal sinusitis. The noninvasive forms are fungal ball (sinus mycetoma) and allergic fungal sinusitis (AFS) (Schubert 2001).

Allergic fungal sinusitis (AFS) is the most common non invasive form of fungal rhinosinusitis. It is a highly recurrent chronic hypertrophic rhinosinusitis that can be distinguished clinically, histopathologically and prognostically from the other forms of chronic fungal rhinosinusitis Patients with (AFS) are immunocompetent, atopic to aeroallergens including the involved fungal organism, have nasal polyps and chronic allergic rhinosinusitis, often produce nasal casts and may occasionally present with proptosis from orbital extension of disease. (Schubert. 2004).

The constellation of characteristics that were described by katzenstein and colleagues in 1983 was the first approach to this newly recognized form of chronic rhinosinusitis at that time. The diagnosis was based on the histological triad of:

1) Clumps or sheets of necrotic eosinophils.
2) Charcot- leyden crystals (from degraded eosinophils).
3) Non invasive fungal hyphae with morphology consistent with

Aspergillus species within the nasal mucus. This gave rise to a descriptive nomeclature “Allergic Aspergillus sinusitis
(Katzenstein et aL, 1983).

Based on the clinical findings in 16 patients, Bent and Kuhn proposed 5 criterias for the diagnosis of AFS:

1) nasal polyposis; (2) allergic mucin; (3) computed tomographic (CT) scan findings consistent with CRS; (4) positive fungal histology or culture and (5) type I hypersensitivity (atopy) diagnosed by history, positive skin tests or serology (Bent and Kuhn 1994).

De Shazo and Swain in 1994, elaborated the diagnostic criteria for AFS which were: sinusitis of one or more pasanasal sinuses on x-ray film; identification of allergic mucin by rhinoscopy or at time of sinus surgery or subsequently on histopathological evaluation of material from the sinus; demonstration of fungal elements in nasal discharge or in material obtained at time of surgery by stain or culture, absence of diabetes, previous or subsequent immuno deficiency disease and treatment with immunosupressive drugs. They excluded atopy as a diagnostic criteria and their cultures yield was only 35% positive
using Sabraud Dextrose Aear medium for culturing the nasal
W W
discharge obtained by swaps from the nasal cavity. (De Shazo and Swain 1995).

Recently, Fonikau and colleagues were able to grow fungal cultures in (202) 96% of 210 patients with CRS with or without polyposis, through

a novel method of nasal mucus collection and a new culturing technique for fungi they also proposed an important criterion for diagnosis of AFS which is the presence of the so- called allergic mucin (clusters of necrotic eosinophils in the mucus). They found the allergic mucin in 96% of 101 CRS patients undergoing endoscopic surgery by histologic examination.

in their study they were able to grow a total of 541 positive cultures using SDA medium and an inhibitory mold agar medium containing cyclohexamide with an average of 2.7 organisms and a maximum of 8 different organisms per patient, a total of 40 di fferent genera of fungi were identified thirty one species have not been associated with or described in AFS before to our knowledge. Interestingly, the control group of normal healthy volunteers (N = 14) was 100% culture positive for fungi thirty-one fungus positive cultures grew with on average of 2.3 different organisms per volunteer and a maximum of 4 different organisms per subject. Eight genera were identified .The organisms grown from the controls were not markedly different than those from CRS patients.

Of the 101 CRS patients undergoing endoscopic surgery fungal elements (hypae, destroyed hyphae, conidiae and sopers)

were found in 82 histological specimens (81%) the allergic mucin was found in 97 (96%) of the 101 consecutive histological specimens.

All together, APS was diagnosed in 93% of their consecutive patients with CRS. These data indicated that the diagnostic criteria for AFS are present in the majority of patients with CRS with or without polyposis. Based on their immunological work up, these authors also postulated that the presence of eosinophils in the allergic mucin occurs independently from a type 1 hypersensitivity and thus should be termed (eosinophilic mucin). This characteristic mucin is propably the common denominator in the pathophysiology of AFS therefore they proposed a change in terminology from (AFS) to “Eosinophilic Fungal Rhinosinusitis” (Ponikau et al., 1999).

A recent study was performed in Graz (Austria) to verify the Mayo clinic report and define the prevalence of eosinophilic fungal rhinosinusitis in Europe employing the same methods of collection and culturing techniques. In this Graz study positive cultures for fungi were obtained in 84 of 92 (91.3%) of their patients with CRS, with an average of 3.2 different species per patient. In all, 33 different genera of fungi were identified with a range of 1 to 9 different species per patient.

In 21 of 23 (91.3%) healthy control subjects, 71 fungus positive cultures grew, with an average of 3.1 different species per volunteer and a range of 1 to 9 different fungi per subject. Sixteen genera were identified.

Of the 37 patients undergoing endoscopic surgery, fungal elements (hyphae, destroyed hyphae, conidia and spores) were found in 28

histological specimens (75.5%) eosinophilic mucin, containing clusters (or sheets) of necrotic eosinophils was found in 35 of 37 (94.6%) histological specimens: overall the criteria of AFS were present in 33 of 37 (89.2%) of their patients undergoing endoscopic surgery. (Bruan et aL, 2003).

In our present study, we applied the same collection and fungal culturing techniques in 100 CRS patients with polyposis and 50 of the controls of normal healthy volunteers (more than double the number of the control group in each of Mayo clinic(Ponikau et ah,1999) and Graz (Bruan et ah,2003) studies, with the following results:

- Cultures were 100% positive for CRS patients and the control group using the Sabaroud Dextrose Agar (SDA) medium.
- Cultures were 98% positive for CRS and the control group using the SDA, chloramphenicol and Gentamycin medium.
- Cultures were 83% positive for CRS patients and 47% positive for the control group using the SDA and Cyclohexamide medium.

A total of 281 positive cultures grew using the three culture media, showed an average of 2.8 organisms and a maximum of 5 organisms per patient, a total of 29 different genera of fungi were identified.

In our control group, a total of 140 fungus positive cultures grew using the three culture media, showed an overage of 2.8 different organisms per volunteer and a maximum of 4 different organisms per subject. The organisms grown from the control were not markedly different as regard type than those from the CRS patients.

In this study fungal elements (hyphae, destroyed hyphae, condidia and Spores) were found in only 60% of histological specimens obtained from the patients undergoing endoscopic surgery when using the GMS and PAS stains. This figure was furtherly recalculated after using the fungalase stain for all specimens stained negatively on GMS staining. As the fungalase stained specimens (N = 40) were 100% positive for fungi hence the overall result was 100% positive.

A comparison of the results of this present study to both the Mayo clinic and the Graz studies' results and the statistical analysis of the mycological and histopathological results among our patients and controls lead to the following data:

- There is difference in the fungal genera that resulted from culturing of the nasal wash of the AFS patients among the three studies; where in both the Mayo clinic and the Graz studies the dematiaceous fungi particularly the Alternaria species were more prevalent unlike in the present study where the Aspergillus species were the significantly predominant organisms.

- Statistical analysis of the commonly encountered fungal species among our present patients versus controls study resulted in being highly significant with a P. value of (0.001) for the Alternaria species, insignificant with a P. value of (0.817) for the Aspergillus niger species and insignificant with a P. value of (1.000) for the

Aspergillus fumigatus species.

- The number of positive cultures among our present patients versus controls study using the three culture medias was insignificant for the SDA and The SDA , chloramphenicol and Gentamycin while in the SDA and cyclohexamide the P. value was (0.002) (highly significant)

- The average number of organisms among our present patients versus controls study using the three culture media was highly significant in both Sabaroud Dextrose Agar (SDA) and SDA, chloramphenicol and Gentamycin with a P. value (0.001) and (0.003) respectively in the SDA and cyclohexamide the P. value was 1.000 (insignificant).

The analysis of the above mentioned data of our present study gives rise to the following facts:

- Although the Aspergillus species where merely more prevalent rather than any other fungal genera in both patients and controls yet the statistical analysis of the most commonly encountered fungal species among patients versus controls proved that Alternaria species is more prevalent among patients rather than controls the matter that may raise the case against Alternaria species as the role causative organism in patients having AFS.

- The SDA and cyclohexamide medium provided the best chance for defining and comparing the different fungal species encountered in the patients versus controls; hence it should be used in every AFS culture.

- Also there is close similarity of the number and types of organisms between both patients and controls. However the average number of organisms was higher in the patients group than in the controls. A matter that should draw our attention to the fact that the disease may be related to the amount of the fungal burden present in the mucus and not the mere presence of fungi.

- Statistical analysis of the histopathological results in the present study directed our attention that the fungalase stain has 100% sensitivity compared to the 60% sensitivity of GIVIS & PAS stain in identifying fungal elements in this study, the 81% sensitivity in the Mayo clinic study and the 75.5% in the Graz study this fact conjugate fungalase staining with every AFS suspected specimen.

CONCLUSION

We have confirmed the presence of allergic mucin in (92%) of our patients having CRS with polyposis by means of histologic examination of the tissue removed during nasal surgery of the 100 consecutive patients. In the same study we have found fungal organisms in 100 **(100%)** of 100 consecutive patients, as examined on the basis of culture and histological examination of the nasal mucus of our patients having CRS with polyposis, and the preoperative CT scan examination have demonstrated features of APS (hyper attenuation foci, polyposis, bony expansion or erosion) in **(**100**%)** of our patients.

Since the major markers of AFS are the striking numbers of eosinophils in the nasal mucus of the patients producing the allergic mucin (clusters of necrotic eosinophils in the mucus), in contrast to the near absence of eosinophils in healthy controls, the presence of non invasive fungal hyphae as evidenced by histological examination or culture and the hyper attenuation foci evident on CT scan.

Our data show that the postulated criteria for the diagnosis of AFS were present in 97% of CRS with polyposis, suggesting that fungi are involved in the disease process of every CRS patient and undoubtedly, the biggest problem facing previous investigators has been the inability to demonstrate fungal organisms in the nasal mucin.

Although, to our surprise, fungal organisms were also detected in the nasal mucus of the majority of healthy control subjects and the organisms grown from the controls were not markedly different than those from the CRS patients. However the average number of organisms was higher in the patients group than in the controls. A matter that should draw our attention to the fact that the disease may be related to the amount of the fungal burden present in the mucus and not the mere presence of fungi giving rise to the future role of the quantitative analysis of the fungal DNA in chronic rhinosinusitis using polymerase chain reaction.

This study has verified that there is geographical variation with regard to the predominantly cultured fungi in AFS patients, where the Aspergillus species particularly the Aspergillus niger were the significantly predominant organisms among both patients and controls. This is at variance with the experience from North America and Europe, where the dematiaceous fungi particularly the Alternaria species were more prevalent.

On the other hand, although the Aspergillus species where merely more prevalent rather than any other fungal genera in both patients and controls yet the statistical analysis of the most commonly encountered fungal species among patients versus controls proved that Alternaria species is more prevalent among patients rather than controls, the matter that may raise the case against Alternaria species as the role causative organism in patients having AFS.

In this study fungal elements (hyphae, destroyed hyphae, condidia and Spores) were found in only 60% of histological specimens obtained from the patients undergoing endoscopic surgery when using the GMS and PAS stains. This figure was furtherly recalculated after using the fungalase stain for all specimens stained negatively on GMS staining. As the fungalase stained specimens (N = 40) were 100% positive for fungi hence the overall result was **(100%)** positive for fungi. The conclusion we draw from this is that the inability to detect fungal organisms in the eosinophilic mucin in prior studies demonstrates the limitations associated with the Grocott methanamine silver stain and that the chitinase-staining technique has greater sensitivity and specificity in detecting fungal organisms within mucin of the patients.

Eventually, our increased awareness towards the rising role of the fungal etiology implementing an allergic immunologic reaction in all cases of chronic rhinosinusitis with bilateral polyposis may lead us to a totally different concept in the management of chronic rhinosinusitis as it would guide the surgeon on the surgical approach, the extent of resection, and any perioperative adjuvant medical therapy including immunotherapy and local antifungal therapy.

SUMMARY

Summary

The combination of nasal polyposis, thick tenacious mucin, crust formation and sinus cultures yielding "Aspergillus" was first noted by *Safirstein, 1976*, who observed the clinical similarity that this constellation of findings shared with allergic bronchopulmonary Aspergillosis. Reports that followed further supported the existence of this clinical entity, and gave rise to descriptive nomenclature such as "allergic Aspergillosis of the Paranasal sinuses","allergic fungal rhino sinusitis", and most recently "eosinophilic fungal rhino sinusitis".

Typical patients with allergic fungal rhino sinusitis are immune competent young adults with recurrent chronic sinusitis, massive nasal polyposis, and thick tenacious mucin in the nose and sinuses. They may have history of multiple sinonasal surgical procedures or history of documented atopic disease, peripheral eosinophilia and elevated total IgE level.

Most authors agree that AFS is an underdiagnosed entity and that only an increased awareness among physicians to look for fungal involvement will increase the accuracy of diagnosing AFS. Unfortunately, previous diagnostic methods seem to lack sensitivity. For example, in the past, even when fungal hyphae were clearly identified in histologic specimens, only 60% of the cultures were positive for fungi.

The aim of this work was to define the true incidence of fungal elements in the nasal and sinus mucin in cases of chronic rhino sinusitis (CRS) with bilateral polyposis compared with normal controls via mycologic and histologic identification of the various fungal species colonizing the viscid allergic mucin.
100 Patients with the clinical diagnosis of chronic rhinosinusitis (CRS) with massive or recurrent bilateral nasal Polyposis were investigated in this

study and compared to 50 Volunteers with no history of nasal or paranasal sinus disease, with no symptoms of inhalant allergy, and with normal appearing mucosa.

Awareness that fungi are colonizing the mucus prompted development of a simple noninvasive procedure to obtain as much mucus as possible for cultures in the same manner the principle of maximum mucus preservation was adhered to during the acquisition of surgical specimens for the histopathological examination and a new immunofluoresent stain (Fungalase) was used in identification of funai.

We have confirmed the presence of allergic mucin in (92%) of our patients having CRS with polyposis.In the same study we have found fungal organisms in 100 **(100%)** of 100 consecutive patients, and the preoperative CT scan examination have demonstrated features of AFS (hyper attenuation foci, polyposis, bony expansion or erosion) in (100%) of our patients. Our data show that the postulated criteria for the diagnosis of AFS were present in 97% of CRS with polyposis, suggesting that fungi are involved in the disease process of every CRS patient.

This study has verified that there is geographical variation with regard to the predominantly cultured fungi in AFS patients, where the Aspergillus species were the significantly predominant organisms among both patients and controls. This is at variance with the experience from North America and Europe, where the dematiaceous fungi particularly the Alternaria species were more prevalent.

Appendix

	Mayo clinic (Ponikau et ah,1999)		Graz (Bruan et al.,2003)		This study	
	patient	Control	patient	Control	patient	Control
Number of cases studied	210	14	92	23	100	50
Number of positive cultures using (SDA and cyclohexamide culture)	202	14	84	21	83	47
Total no. of positive cultures Using the three medias	541	42	290	63	281	140
Average no. of organism per patient.	2.7	2.3	3.2	3.1	2.8	2.8 ;
Maximum no. of organism per patient.	8	4	9	9	5	4
Number of genera identified	40	8	33	16	29	25
No. of positive histopathologic specimens.(fungal element)	82	not done	28	not done	100	not done
No .of histopathologic specimens containing eosinophilic mucin	97	not done	35	not done	92	not done

Table 16: Comparison of the results of the studies of Mayo clinic, Graz versus our present study.

Table 17 : Comparison of the fungal genera recovered from the patients in Mayo clinic study, Graz study versus the results of our present study.

Fungal genera)	No. of positive cultures (Mayo clinic N=210)	No. of positive cultures (Graz N=92)	No of positive cultures (this study N=100)
Acremonium	5	0	1
Alterncíria	93	61	28
Ampelomyces species	0	6	0
Aporospora species	0	1	0
Art hr in ium species	0	1	0
Arachniotus ciirimis	3	0	0
Arthrographis- kalrae	1	0	0
Aspergillus	62	77	114
A. clavatus	0	1	0
A. fischerianus	0	1	0
; *A. jlavits*	8		17
A. fwnigains	17	30	28
... — ■	6	0	0
A. niclulcms	1	11	4
A. niger	5	2	51
A. rubrobrunneus	0	2	0
A.tetrazonus	0	1	0
A. terreus	2	0	2
A. versicolor	15	0	10
A. vers (forme	1	0	0
A. species	7	26	4
Aureobasidium	8	11	1
!..... " ---- *Basidiomcota*	0	12	0
Beauveria	2	2	0
: *Bipolaris*	2	0	5

Table 17 : Comparison of the fungal genera recovered from the patients in Mayo clinic study, Graz study versus the results of our present study.

Fungal qenera	No. of positive cultures (Mayo ciinic N=210)	No. of positive cultures (Graz N-92)	No of positive cultures (this study N=100)
Bjerkandera adusta	0	1	0
Boirytis cinerea	0	1	0
Candida	45	8	6
C *albicans*	31	6	6
C. glabrata	0	1	0
C. krusei	1	0	0
C. lipolytica	1	0	0
C, lusitcmiae	1	0	o
C. parapsilosis	9	1	0
C. tropical is	1	0	0
Candida species		0	0
Chaetominm	3	1	1
Chryosporium	2	0	4
Clcidosporium	82	38	8
Cryptococcus	4	0	0
C alhidns		0	0
C laurentii	2	0	0
Cryptococcus sp.		0	0
Ciirvularia	2	1	8
Dactyl aria spp.	0	0	4
i *Dematiaceous fungus*	0	0	1
Echinodontium \ taxodi	0	1	Q
\ *Epicoccum*	12	2	3
Exophiala jeanselmci	2	0	0

fable 17 : Comparison of the fungal genera recovered from the patients in Mayo clinic study, Graz study versus the results of our present study. (Continued)

Funqal genera	No. of positive cultures (Mayo clinic N=210)	No. of positive cultures (Graz N=92)	No of positive cultures (this study N=100)
F mar ium	34	2	4
Geotrichum	10	0	0
Gliomastix	1	0	0
Heterobasidion annosum	0	2	0
Hormonema dematioides	0	5	0
Hypocrea schweinitzii	0	2	0
Leptosphaerici microscopia	0	2	0
Mon ilia	3	0	0
Mucor	4	0	5
Nigrospora	1	0	2
Oidiodendron	1	0	0
Paecilomyces	5	2	0
Papular ia	4	0	0
Penicillium	91	52	14
Phoma	2	2	0
Pithomyces	14	0	4
Plea spar a papaveracea	0	2	0
■ *Psen da lies c her ia~ boydii*	1	0	22
Rhinocladiella	3	0	0
Rhizopus	5	0	1
Rhodotorula	4	0	0
R minuta	2	0	0
Rhodotorula sp.	2	0	0

Table 17 : Comparison of the fungal genera recovered
patients in Mayo clinic study, Graz study versus the r
our present study. (Continued)

Funqa! qenera	No. of positive cultures {Mayo clinic N=210)	No. of positive cultures (Graz N"92)	No of positive cultures (this study N=100)
Saccharomyces cerevisiae		0	0
Sagrahamala	1	0	0
Scolecobasidium	1	0	0
Scopnlariopsis	3	0	0
S. brumptii	1	0	0
Schizophyllum			
commune	0	3	0
Sclerotinia sclerotiorum	0	10	0
Scopnlariopsis	2	0	0
Stachybotrys	0	0	9
Thanatephorus cucumeris	0	2	0
Trichciptum fusco-violaceum	0		0
Trichoderma	8	3	4
Tricholoma robustum	0	2	0
Trichophyton	2	0	0
T riibrum	1	0	0
Trichophyton sp.		0	0
Trichosporon-beigelii	1		1
Ustilago	13	1	
Total No. of organisms	541	290	281

Table 17 : Comparison of the fungal genera recovered i
patients in Mayo clinic study, Graz study versus the re

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